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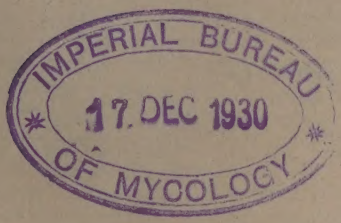
A STUDY OF MEADOW-CROP DISEASES IN NEW YORK

JAMES G. HORSFALL



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A STUDY OF MEADOW-CROP DISEASES IN NEW YORK[1]

JAMES G. HORSFALL

OBJECT OF THE INVESTIGATION

A paucity of information, especially that which is easily accessible, led to an investigation of meadow-crop diseases. In studying such a wide range of diseases, the writer accumulated an extensive bibliography that has made possible the bringing together of the important information extant on the diseases of meadow crops. No claim to completeness of bibliography is made since many papers, which for various reasons were not found in indices, abstract journals, or other papers, are missing.

Briefly, the objects of the work have been: (1) to study methods of determining loss from foliage troubles, (2) to describe the diagnostic characters for the diseases, (3) to untangle the nomenclature of the causal organisms, (4) to make observations and experiments upon life history, especially the factors governing spore germination, and (5) to determine the effects of sulfur and copper dusts in combating these diseases.

The pioneer work of Kightlinger (1925) and the later work of Greaney (1928) and of others in controlling wheat rust successfully with fungicides stimulated the investigation looking toward a similar control for meadow-crop diseases. This study has centered around laboratory tests of the effect of fungicides upon germinating spores, using methods long applied in the laboratory of Plant Pathology at Cornell University and described recently by McCallan (1930). The laboratory tests were supplemented by greenhouse and field studies, although the latter was limited by the facilities available. As McCallan has pointed out, the results of laboratory tests may be used as reliable indicators of the efficiency of fungicides in controlling diseases. Laboratory tests serve admirably to narrow the scope of field work which, of course, will furnish the ultimate criterion of the value of the fungicides.

The diseases of the three clovers, red clover, *Trifolium pratense* L., alsike clover, *T. hybridum* L., and white clover, *T. repens* L., and of timothy,

[1] Also presented to the Faculty of the Graduate School of Cornell University, February, 1929, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

AUTHOR'S ACKNOWLEDGMENT. The author wishes to express his appreciation for many helpful criticisms and suggestions to Professor H. H. Whetzel, who suggested the problem and under whose immediate direction the work was done. He wishes also to acknowledge the assistance rendered by Professor M. F. Barrus and the other members of the staff of the Department of Plant Pathology, Cornell University, who have rendered assistance of various kinds.

Phleum pratense L., have been studied most intensively, and some attention has been paid to the diseases of the less-important clovers and grasses in the State. Some consideration has been given to a destructive disease of blue-grass, *Poa pratensis* L., which, though not a crop of first rank in the State, is commonly found on lawns, golf courses, and in meadow mixtures. Many pathogenes attacking the first-named suscepts have been studied also in their relation to other possible suscepts. The diseases of alfalfa, *Medicago sativa* L., were included in the survey of the State, but a further study of them was not made unless the plant happened to be a suscept of some of the other diseases involved in the investigation. The following is a list of the other suscepts mentioned in this paper: crimson clover, *Trifolium incarnatum* L.; sweet clover, *Melilotus alba* Desr.; zigzag clover, *Trifolium medium* L.; hop clover, *Trifolium agrarium* L.; vetch, *Vicia villosa* Roth.; yellow trefoil, or black medick, *Medicago lupulina* L.; red top, *Agrostis alba* L.; orchard grass, *Dactylis glomerata* L.; quack grass, *Agropyron repens* (L.) Beauv. The writer has followed Wiegand and Eames (1925) in the use of Latin names of the suscepts found in New York.

IMPORTANCE OF MEADOW CROPS IN THE STATE

An investigation of the diseases of meadow crops in New York is particularly important since hay is a valuable crop in the State and since New York leads the Union in its production. According to the *New York State Crop Report* for January, 1928, published by the New York State Department of Agriculture and Markets, the tame-hay crop of the State in 1927 was worth $82,614,000. This is more than twice the value of the potato crop, the nearest competitor, which is listed at $35,775,000. The hay crop in 1927 was worth more than the combined valuation of potatoes, corn, apples, and oats, which are the crops of next importance arranged in descending order. It was worth also twice as much as all the following crops combined: winter wheat, barley, beans, buckwheat, cabbage, grapes, pears, strawberries, and cauliflower, each one of which is valued at more than $2,000,000.

In the United States as a whole, hay ranks third in value as a crop, being exceeded only by corn and cotton. It, therefore, is obvious that an investigation of the diseases of the hay crop is not without economic significance. This is especially true in New York, which leads the Nation in hay production, both in acreage and in tonnage (Callander and others, 1927:986). In 1926, New York planted a timothy and clover mixture on 2,205,000 acres and harvested 2,999,000 tons of hay. Her nearest rival in hay production is Wisconsin, where the 1,710.000 acres planted in 1926 yielded 1,650,000 tons of hay.

SURVEY OF THE MEADOW-CROP DISEASES OF THE STATE

THE NECESSITY OF A METHOD FOR DETERMINING LOSS

The necessity of having a satisfactory method for determining which are the important diseases, and for determining their distribution and their severity, has been impressed upon the writer repeatedly during the past three years. Pathologists have worried long over the problem of determining the importance, both relative and absolute, of leaf and stem diseases. Tehon (1927) has made an interesting study of losses caused by cereal diseases, but his method seems laborious. In 1928 an investigation of methods for determining the losses from meadow-crop diseases was undertaken. The writer realizes that the method described herein fails to fulfill all the requirements of perfection, but it is presented in the hope that it may be a contribution toward that end. The technic was developed as fully as the time available permitted. First, it was determined where the most hay was grown; then, during June, just prior to the first cutting, a survey trip was made through these regions, stopping frequently and determining as nearly as possible what diseases were present and how destructive they were. Thus a random sample of the meadow-crop diseases of the State was procured which served as a basis for arranging the diseases in the relative order of their importance and for calculating the losses that they bring about. During the course of the work 194 fields in 35 counties were examined. As a rule, no two stops were made within five miles of each other.

DISTRIBUTION OF MEADOW CROPS

Method of determining meadow-crop distribution

The distribution of hay crops in the State was obtained from the 1925 Agricultural Census by calculating the percentage of crop land, by counties, devoted to each kind of hay — timothy alone, timothy and clover mixed, clover alone, and alfalfa alone. Because timothy alone in a meadow results from the gradual dying out of clover in the mixture and because clover is rarely planted by itself, the percentages of crop land devoted to a mixed stand of timothy and clover, as shown in figure 1, are taken as a fair index of the distribution of cultivated hay, other than alfalfa, in the counties of the State. The survey itinerary was laid out with these points in mind. The percentage of crop land devoted to hay is somewhat misleading in some instances, a marked case being Sullivan County in the Catskill Mountains where the amount of crop land is very limited.

Method of gathering data in the field

The following method of taking data finally was adopted. It gives a fairly quantitative measure of the disease and at the same time requires

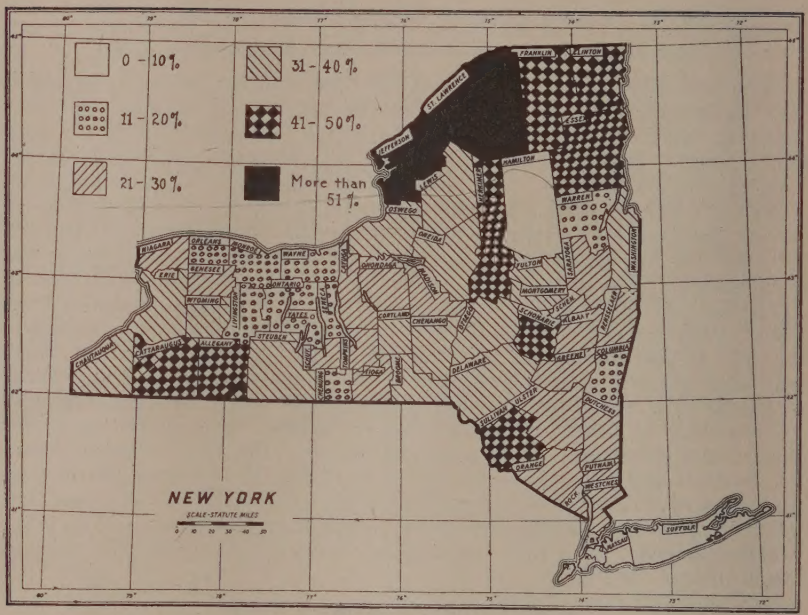


FIGURE 1. PERCENTAGE OF CROP LAND IN NEW YORK DEVOTED TO TIMOTHY AND CLOVER MIXTURE

a minimum expenditure of time in each field. It is essentially the same as that used by Köck (1927) in determining the susceptibility of apple varieties to powdery mildew.

The following classification of the amount of disease present was used: heavy, moderate, light, a trace, and no infection. The limits in the percentage of diseased leaves or stems for each group, depending upon the nature of the malady, were arbitrarily established as follows: heavy, from 65 to 100 per cent; moderate, from 25 to 65 per cent; light, from a trace to 25 per cent; trace, only a few scattered cases in a field. At first these limits were founded upon actual counts of each disease in each field, but after the groups had been used a short time, ability to estimate the percentages rather accurately was attained. The limits of each group are widely separated, to be sure, but since a great many factors, such as local rainfall, air and soil drainage, density of stand, variety, and soil types, jointly determined the amount of infection, the method probably is as useful as that of making several counts of each disease in each field.

In illustrating the method of assembling data, suppose a single suscept, red clover, be used. The data for each disease are arranged in a fraction, the "index of infection." The total number of fields of red clover observed is the denominator, which is the same for all diseases of this suscept. Each of the five groups of infection is represented by a bracket of figures in the numerator, the figures showing the percentage of cases in which the amount of infection has been placed in that group. For example, Pseudopeziza leaf spot has been found to be represented by the fraction $\frac{(3)\ (24)\ (42)\ (13)\ (18)}{119}$. From this fraction, it is readily concluded that red clover was observed in 119 fields; the disease was adjudged heavy in 3 per cent of the cases, moderate in 24 per cent, light in 42 per cent, a trace in 13 per cent, and 18 per cent of the fields showed none of it. If, therefore, the numerator be large toward the left side, the disease is severe in the State; but if large toward the right side, the disease is relatively unimportant. Unfortunately, the fractions may not necessarily be directly comparable. Anthracnose is a case in point. It has the following fraction: $\frac{(1)\ (9)\ (33)\ (15)\ (42)}{119}$. Hence it may be said to be not so serious as the Pseudopeziza leaf spot. Such a conclusion probably is fallacious in as much as the anthracnose organism attacks stems and tends to stunt or kill parts above the lesion, whereas Pseudopeziza causes only local injury to the leaves.

It became more and more evident as the work progressed that the selection of the grouping method was a happy one, for in all fields in the same group approximately the same ratio appeared to exist between the percentage of healthy leaves, the percentage of leaves in various stages of disease, and the percentage of dead leaves. If, for example, 60 per cent of the leaves were infected, then about 15 per cent of these would be dead, and the others would lie along an infection gradient from severely infected to healthy. If such a relation held true, then a method of measuring the loss, say in a field rated as moderately infected, should be applicable to the other groups as well. But to find such a method — that was more difficult.

ECONOMIC IMPORTANCE

Nature of loss

Leaf-spot diseases of meadow crops induce loss (1) by lowering the quality or palatability of the affected tissues, (2) by reducing the carbohydrate-producing area, (3) by causing defoliation during growth, and

(4) by increasing the percentage of leaves that shatter during harvest. The operation of the second and third factors mentioned results in subsequently diminished growth and a lowered quant ty of sugars and other carbohydrates available for food in the hay. Stem and root diseases cause more profound injuries than do leaf-spot diseases, because they attack the supporting and conduct on systems of the plants, resulting in loss of vitality, in wilting, and in stunting.

Amount of loss

Method of determining relative importance

The acquisition of an index of infection for each disease places the investigator in a position to determine the relative importance of the diseases.

Method of determining absolute loss

The index of infection was used as a basis for calculating the absolute loss caused by these leaf-spot diseases. Methods involving the measuring or weighing of plants from representative fields of each infection group were abandoned because variations in soil, stand, age of stand, age of plants, varieties, air and water drainage, as well as inaccuracy of balances with pans large enough to hold a plant, all function jointly in making such methods valueless.

It thus was essential to make all measurements in the same field and, if possible, on the same plants. The possibility of leaf measurements presented itself in this connection. A field of red clover in which about 45 per cent of the leaves were diseased, and was therefore moderately infected with *Macrosporium sarcinaeforme*, was chosen for these leaf measurements. The disease in this field seemed as nearly ideal as possible for study because (1) the clover was relatively free of other pathogenes; (2) the area of the leaf spots, which were practically circular, was easy to determine by averaging two diameters; and (3) the clover, which was growing rapidly, gave a fair amount of foliar material on each plant with which to work.

Plants picked at random five paces apart were brought to the laboratory and were placed in water. The number of dead leaves was noted, the area of each living leaf was found with a planimeter, and the spots on the leaf were classified according to size in thirteen groups ranging from 1/50 up to 13/50 inch in diameter. From these data the total leaf area, the total spotted area, and the percentage of spotted leaf area for each plant could be calculated.

The figures thus obtained are set forth in table 1.

TABLE 1. DETERMINATION OF DISEASED AREA OF RED-CLOVER LEAVES AFFECTED BY MACROSPORIUM SARCINAEFORME

Plant no.	Number of leaves dead	Total number of leaves	Area in living leaves (square inches)	Area spotted (square inches)	Area spotted (per cent)	Total diseased area (per cent)*
1	7	34	73.696	1.9261	2.61	22.66
2	2	9	8.416	0.1727	2.05	23.82
3	7	14	7.360	0.0345	0.46	50.23
4	19	31	17.088	0.2573	1.51	61.88
5	15	35	34.432	2.6413	7.67	47.24
6	3	13	14.784	0.2793	1.89	24.53
7	3	16	14.448	0.3479	2.41	23.66
Average	..	..	.....	.....	2.66	36.30

* This last column of the table, which shows the percentage of total foliar area diseased, was calculated by assuming the area of each dead leaf to be equal to the average area of the living leaves. Then the total area of dead leaves is added to the total area in spots, this is divided by the total area of all leaves, and the quotient is expressed in a percentage.

It may be argued that some leaves are killed by shading, but where no leaf spots are present, fields show almost no dead leaves even though the stand be exceedingly dense. Apparently, healthy clover leaves are very resistant to shading. In the field concerned in the measurements, however, the stand was thin enough so that shading was reduced to a minimum.

If the dead leaves and the dead spots in living leaves be charged against the pathogene and considered a total loss to the grower, then he lost approximately 36 per cent of the leaf material in that clover crop. According to Piper (1921:398), clover hay by weight consists of about 60 per cent stems, 30 per cent leaves, and 10 per cent flower heads. If this be true, then every ton contains 600 pounds of leaves. Since 36 per cent of this is destroyed (table 1), approximately 216 pounds out of every ton was lost from this field of red clover. This was a loss of material that contains about three times as much protein as do the stems. It is true that the leaf loss is not total since most of the dead material is present, but this is counterbalanced on the other hand by (1) decreased growth additions of stems, leaves, and flowers, (2) lowered quality, and (3) increased shattering during harvest. For the purposes of this paper, therefore, the assumption is made that a clover leaf-spot disease of moderate intensity reduces the leaf yield by a third, and this reduces the yield of the entire crop by about 11 per cent. Since 45 per cent of diseased leaves, an average for the moderate class, causes a total loss of 11 per cent, then each per cent of diseased leaves causes a loss of 11/45, or 0.25 per cent, in round numbers.

This calculation probably tends to be more conservative than otherwise. If the spots appear early in the life of the plant, they will cause

more profound effects than if they appear late. In as much as this effect is extremely difficult to evaluate, the calculation has not been altered to account for it.

In the course of the measurement of the diseased leaves, the question arose as to the amount of affected tissue necessary to induce yellowing and death. Obviously, the reactions in each spot affect the physiology of the adjacent tissue, and hence the entire area need not actually be spotted before the leaf dies. The most severely diseased leaf measured in preparing table 1 had 19.82 per cent of its area visibly spotted; another leaf had 17.28 per cent of its area spotted. To determine whether these leaves approached the limit, a number of the most severely spotted unyellowed leaves and severely spotted incipiently yellowed leaves were collected from the same field and were measured as before. These data are presented in table 2.

TABLE 2. PERCENTAGE OF AREA OF RED-CLOVER LEAVES SPOTTED WHEN SEVERELY DISEASED BY MACROSPORIUM SARCINAEFORME

Leaf no.	Unyellowed leaves			Incipiently yellowed leaves			
	Total area (square inches)	Area spotted		Leaf no.	Total area (square inches)	Area spotted	
		Square inches	Per cent			Square inches	Per cent
1	3.920	0.7864	20.06	1	2.240	0.1862	8.31
2	2.448	0.2450	10.01	2	3.872	0.3433	8.90
3	2.448	0.3571	14.59	3	1.728	0.2039	11.80
4	2.960	0.4098	13.80	4	2.288	0.2757	12.10
5	2.368	0.2609	11.02	5	2.704	0.5102	18.90
Average	.....	.....	13.85	.....	.....	.....	12.00

The assumptions in making these measurements are (1) that the incipiently yellowed leaves represent a more advanced stage of laminar infection than do the unyellowed ones; (2) that therefore a larger percentage of their area is spotted; and (3) that a point midway between these two extremes is the very largest percentage of area that could be diseased without killing the leaf. A glance at table 2, however, shows that these assumptions, at least in part, are false, because the unyellowed leaves show more diseased area on the blades than do those just beginning to turn yellow. The reason for the discrepancy fortunately is not far to seek, for the incipiently yellowed leaves almost invariably have lesions on their petioles. No such lesions, however, appear on leaf no. 1 in the table of the unyellowed series or on leaf no. 5 of the yellowed series. These petiolar lesions induce a general yellowed condition in the blades, causing them to die promptly, whereas blade infection alone does not induce a

uniform yellowing but rather the yellowing spreads outward from the infection foci. A uniform yellowing therefore is an unsafe criterion for determining death of leaves from leaf spots. Since, however, no leaf of the 106 measured shows more than 20 per cent of its area involved, the conclusion is that when more infections occur, the leaf dies and fails to appear on the data sheet.

This conclusion is pertinent to the discussion regarding the appropriateness of the grouping method for determining the degree of severity of the disease, because it gives a reason for the apparent correlation between the number of dead leaves and the number of living leaves on each plant. Since a leaf can withstand only a certain number of infections before it dies, the number of dead leaves should vary directly with the degeree of severity of the disease. A corollary of this conclusion is that the greatest loss arises from leaves killed by the fungus and not from spots in living tissue, even though these spots appear exceedingly numerous to the observer.

Now that each percentage of diseased leaves has been shown to cause 0.25 per cent actual loss, the reduction in yield caused by clover leaf spots in New York can be calculated from the index of infection. Of course, the basic assumption in this calculation is that each organism kills the leaf when approximately 20 per cent or less of its area is involved. This is true within the limits of error of this method of calculation.

The index of infection, the percentage loss, and the loss in tons that have been figured for each leaf-spot disease of clover and alfalfa are arranged in descending order of importance and presented in table 3. The calculation of the percentage-loss column may be illustrated by Macrosporium leaf spot of red clover. The index of infection is $\frac{(0)\ (3)\ (34)\ (25)\ (38)}{119}$. The essential calculation is to find the percentage of diseased leaves, because the loss induced by each per cent is already known. The average percentage of diseased leaves in each group, as defined on page 6, is as follows: heavy, 82 per cent; moderate, 45 per cent; light, 12 per cent; trace, essentially 0 per cent; and none, 0 per cent. If, therefore, the percentage of fields falling into each group be multiplied by the average percentage of diseased leaves in that group, the product will be the percentage of diseased leaves in that group for the State, and the sum of these products will be the percentage of diseased leaves in the entire State (with respect to the disease in question). Thus $(0 \times 82) + (3 \times 45) + (34 \times 12) + (25 \times 0) + (38 \times 0) = 5.43$ per cent of the red-clover leaves in the State diseased with Macrosporium leaf spot. Since each per cent of diseased leaves means an actual loss of 0.25 per cent, this disease diminishes the crop by 1.36 per cent.

Losses induced by diseases in meadows

According to the *New York State Crop Report* (January, 1928), 852,000 tons of clover hay, 3,532,000 tons of clover and timothy mixed, and 569,000 tons of alfalfa were produced in the State in 1927. The approximate total yield of clover for that year, found by adding half the yield of clover and timothy mixed to that of clover alone, was 2,618,000 tons.

The calculation of the loss in tons for table 3 may be illustrated by the Pseudopeziza leaf spot of red clover. A loss of 4.62 per cent means that the total yield for the State of 2,618,000 tons is only 95.38 per cent of what it would be were this disease not present. The total yield is divided by 95.38, the quotient is multiplied by 100, and the total yield is subtracted from the figure obtained, to give the loss in tons. The same method used in calculating the losses from clover diseases is applied to alfalfa diseases because it undoubtedly is better than a guess as to the loss involved.

TABLE 3. SUMMARY OF MEADOW-CROP-DISEASE RECORDS, LEGUMES

Suscept	Fungus or disease	Index of infection	Loss	
			Per cent	Tons
Red clover, 119 fields	Pseudopeziza leaf spot	(3) (24) (42) (13) (18)	4.62	126,810
	Macrosporium leaf spot	(0) (3) (34) (25) (38)	1.36	36,095
	Anthracnose *	(1) (9) (33) (15) (42)	....	.....
	Mosaic †	(0) (0) (6) (07) (87)	0.24	6,298
	Pseudoplea leaf spot	(0) (0) (6) (7) (87)	0.24	6,298
	Cercospora leaf spot	(0) (0) (3) (4) (93)	0.09	2,358
	Rust	(0) (0) (3) (4) (93)	0.09	2,358
Total			6 64	180,217
Alsike clover, 102 fields	Cercospora leaf spot	(0) (2) (35) (24) (39)	1.27	.....
	Rust	(0) (7) (30) (10) (53)	0.81	.....
	Pseudoplea leaf spot	(0) (0) (16) (12) (72)	0.48	.....
	Mosaic	(0) (1) (7) (7) (85)	0.32	.....
	Sooty blotch	(0) (0) (6) (6) (88)	0.24	.....
	Stagonospora leaf spot	(0) (0) (5) (5) (90)	0.15	.....
Total			3.27	.....
Alfalfa, 95 fields	Pseudopeziza leaf spot	(6) (22) (44) (11) (17)	5.12	30,704
	Stagonospora leaf spot	(0) (8) (38) (8) (46)	2.04	11,849
	Root knot (Diplodina)	(2) (7) (7) (3) (81)	....	.....
	White spot	(1) (1) (9) (6) (83)	0.60	3,434
	Pyrenopeziza leaf spot	(1) (0) (3) (1) (95)	0.31	1,769
Total			8 07	47,756
White clover, 14 fields	Pseudoplea leaf spot ‡	(15) (7) (50) (7) (21)	5.59	.....
	Stagonospora leaf spot	(0) (0) (43) (2) (55)	1.29	.....
	Cercospora leaf spot ‡	(0) (0) (36) (2) (62)	1.08	.....
	Rust	(0) (0) (28) (28) (44)	0.84	.....
	Sooty blotch	(2) (2) (4) (2) (90)	0.76	.....
Total			9.56	.....

* Anthracnose is not admissible to the loss calculations which apply to leaf-spot diseases only.
† Mosaic probably is not accurately figured by this method.
‡ Apparently a new record for the State.

According to the estimates of Callander and his associates (1927:991), which are the most recently available data, the farmers of the country received an average of $14.52 a ton for clover hay and $13.52 a ton for alfalfa during 1926. Since the hay prices have not varied widely through recent years, these prices are considered satisfactory for 1928 in New York State. On this basis New York growers actually lost approximately $2,515,000 in 1928 because of disease in their clover and $645,000 on alfalfa, making a loss of approximately $3,160,000 on these two hay crops alone.

This loss came from leaf spots only and does not take into consideration such diseases as anthracnose, powdery mildew, and root rots, which undoubtedly do much additional damage in the State.

From table 3 it appears that Pseudopeziza leaf spot, anthracnose, and Macrosporium leaf spot are the most serious diseases of red clover in the State. Cercospora leaf spot, rust, and Pseudoplea leaf spot attack alsike severely, while Pseudoplea leaf spot and rust seem to be worse on white clover. Among the diseases of alfalfa, Pseudopeziza leaf spot and Stagonospora leaf spot are most destructive.

The diseases observed on grasses and miscellaneous suscepts are brought together in table 4.

TABLE 4. Summary of Meadow-Crop-Disease Records, Grasses

Suscept	Fungus or disease	Index of infection
Timothy *	Cercospora leaf spot	(3) (15) (44) (19) (19)
	Heterosporium leaf spot	(3) (2) (28) (36) (31)
	Ustilago striaeformis	(0) (0) (3) (4) (93)
Blue-grass, 45 fields	Helminthosporium leaf spot	(4) (18) (50) (11) (17)
	Rust	(0) (0) (6) (4) (94)
	Septoria leaf spot †	(0) (0) (4) (2) (94)
	Ustilago striaeformis	(0) (0) (4) (2) (94)
Orchard grass, 14 fields	Cercospora leaf spot †	(0) (28) (50) (2) (20)
	Cercosporella leaf spot †	(0) (0) (7) (15) (78)

* Sixty-four-fields basis for Cercospora estimate, 93-fields basis for the others.
† Apparently a new record for the State.

From table 4 it appears that timothy is most severely attacked by Cercospora leaf spot and next by Heterosporium leaf spot. Cercospora leaf spot is also destructive on orchard grass, while Helminthosporium leaf spot is the most ruinous disease affecting blue-grass.

The miscellaneous, or rare, diseases or fungi that the survey revealed on legumes and grasses are as follows, the apparently new records being marked with an asterisk (*). On red clover: * Stagonospora leaf spot, sooty blotch, * bacterial leaf spot, powdery mildew, * girdle, and * gray

mold of stems caused by *Botrytis* sp.; on alsike clover: * Stagonospora leaf spot, Pseudopeziza leaf spot, and powdery mildew; on white clover: * Pseudopeziza leaf spot, * bacterial leaf spot, * powdery mildew, and mosaic; on alfalfa: yellows, downy mildew, walnut antagonism, Cercospora leaf spot, * Pseudoplea leaf spot, * Macrosporium leaf spot, and * girdle; on sweet clover: Cercospora leaf spot, Stagonospora leaf spot, * girdle, and mosaic: on vetch: *Botrytis* sp. (causing a leaf spot); on hop clover: Cercospora leaf spot; on yellow trefoil: * Cercospora leaf spot, * Stagonospora leaf spot, * Pseudoplea leaf spot, and Pseudopeziza leaf spot; on timothy: rust and *Cladosporium graminum*; on red top: * Cercospora leaf spot, *Helminthosporium triseptatum*, and stem rust; on quack grass (a meadow crop by courtesy): *Phyllachora graminis*, stem rust, *Pyrenophora tritici-repentis* (ascospore and conidial stages), * *Septoria agropyri*, and *Urocystis agropyri*; on foxtail: Piricularia leaf spot and head smut; on orchard grass: stem rust and leaf smut; on blue-grass: powdery mildew and leaf rust.

DISCUSSION OF SPECIFIC DISEASES

The diseases discussed were studied with the following points in mind: (1) to find diagnostic characters for separating them; (2) to unravel the nomenclatorial tangles encountered; (3) to study the life history of the pathogene through field work, pure cultures and inoculations; (4) to study the factors affecting incubation and infection; and (5) to determine the sensitivity of the causal organisms to various dust fungicides, by tests in the laboratory, greenhouse, and field. A discussion of the importance of each disease in the State is usually omitted, because this is indicated in tables 3 and 4. Some information gleaned from the literature on this point, however, is given in certain instances for comparison.

DISEASES OF LEGUMES

MACROSPORIUM LEAF SPOT

Suscepts

No suscept other than red clover has been found naturally affected by Macrosporium leaf spot in New York, but alsike, white clover, alfalfa, and sweet clover have been successfully inoculated by the writer and the pathogene has been reisolated. These plants showed fewer and much smaller spots than those on the red clover inoculated at the same time, indicating that they are less susceptible. Krakover's (1917:289) negative results from inoculation trials upon alsike clover, crimson clover, white clover, and alfalfa may indicate pathogenic differences among various strains of the causal organism. Alsike clover has been reported as diseased

when associated with affected red clover (Bain and Essary, 1905:503). Elliott (1917:457) reports successful inoculation of white clover and alfalfa with a pure culture of Macrosporium isolated from red clover, but he reports no reisolation of his fungus. Several others also have reported red clover as susceptible to this disease in the field (Milburn and Bessey, 1915:72; Westgate, 1908:42; Gentner, 1918; Tehon and Daniels, 1925:714). Many of these writers undoubtedly have confused this disease with a closely related Macrosporium leaf spot occurring on alfalfa.

Gentner (1918) declares that Italian red clover is the variety most susceptible to this disease. Hesler (1925:93) found no appreciable differences in the susceptibility of varieties.

The disease

Many writers have referred to this disease without giving it a specific designation. Stakman (1922:248), however, called it *large leaf spot.* Since this spot is not necessarily large, the name *Macrosporium leaf spot* is proposed to set it off more definitely from other similar leaf spots, especially Pseudopeziza leaf spot with which it is most likely to be confused. Stevens and Hall (1913:390) use the term *Macrosporiose* to denote the same idea.

History and range

Cavara (1890) first discovered this disease near Pavia, Italy, in 1890. It was reported from Germany in 1897 by Tubeuf and Smith (1897:517). Probably the first specimen collected in this country was found on clover by May Varney at Manhattan, Kansas, in 1889 (Tehon and Daniels, 1925:714). The disease has been reported from England by Milburn (1915:72). The trouble occurs in the eastern United States from Maine to Louisiana. The first specimen from the vicinity of Ithaca deposited in the Cornell Plant Pathology herbarium was collected in 1915.

The most recent and complete treatise yet published dealing with this disease is that of Krakover (1917:273–328).

Symptomatology

The lesions of this disease are limited almost exclusively to the leaf blades, although they sometimes occur on stems and petioles, where they appear as minute black streaks from 1 to 3 millimeters in length. Petiolar lesions cause the leaf to yellow prematurely. Milburn (1915:72) says that the disease affects the seeds, which are shrunken and shriveled and much darker than healthy seeds. In young stages the leaf spots are minute and light brown in color, but they enlarge rapidly, coalesce, and kill the blade when approximately 20 per cent of its area is affected. The char-

FIGURE 2. SPOTS ON LEAVES OF RED CLOVER CAUSED BY MACROSPORIUM SARCINAEFORME

acteristic concentric zones of full-sized spots, as shown in figure 2, make the disease almost unmistakable in the field. In young stages the spots are extremely difficult to distinguish from those caused by Pseudopeziza, but a careful examination usually will reveal a hint of the zonation. The older Pseudopeziza spots bear in their center the diagnostic sign of that disease, the minute dried apothecium. The latter spots are frequently dendritic in outline, but such a characteristic never occurs with the Macrosporium leaf spot.

Etiology

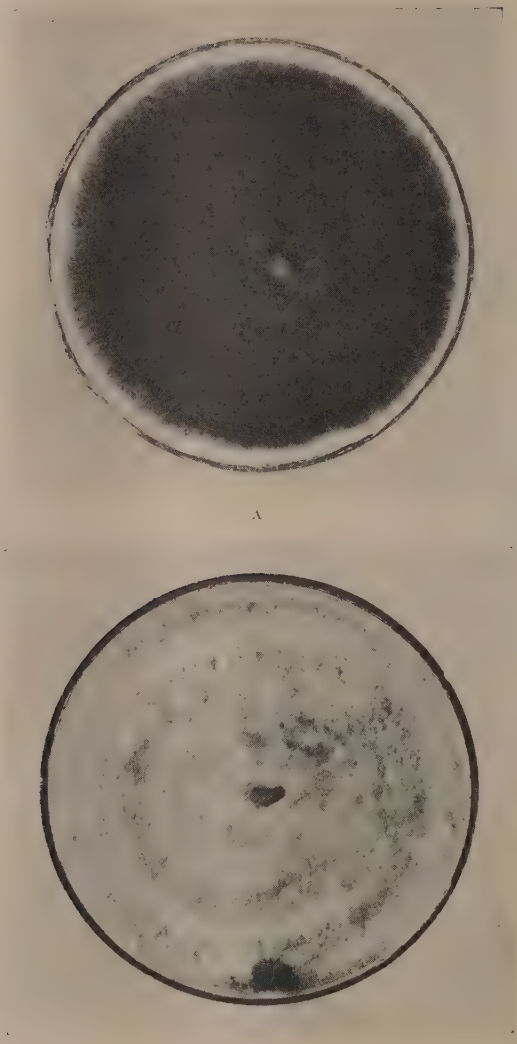
Name, history, and classification of the pathogene. The pathogene was first described by Cavara (1890) at Pavia, Italy, as *Macrosporium sarcinaeforme*. Twenty-five conidia from one specimen collected at Ithaca measured 23.4 to 32.4 μ (mean 28.01 $\pm$ 0.02ò) by 19.8 to 25.2 μ (mean 23.39). The original measurements of Cavara are 24 to 28 μ by 12 to 18 μ. Krakover (1917:328) measured many spores from several different pure

cultures and found that they varied from 22.4 to 37.7 μ by 19.1 to 27.4 μ. The writer's measurements fall within the latter limits.

The name of the fungus has been juggled somewhat in the literature because of a misunderstanding of the perfect stage and because of the confusion of the two fungi on clover and alfalfa. Gentner (1918) reports perithecia of *Pleospora herbarum* Rabenh. in pure cultures of a fungus which he identified as *M. sarcinaeforme*. Marchal and Marchal (1921) state that *Pleospora lycopersici* n. sp. is the perithecial form *M. sarcinaeforme*. Krakover (1917:279) concludes that the fungus on red clover is different from that on alfalfa because the conidia of alfalfa are much smaller and decidedly warty, whereas he had never seen any but smooth spores from red clover. Gentner (1918) also finds some slight morphological differences between the fungi on the two suscepts; but since conidia from different varieties of alfalfa also differ, he concludes that the two are identical. Tehon and Daniels (1925:717) following Elliott's (1917:471) suggestion that the fungus is not a good member of the genus Macrosporium, and assuming that the forms on clover and alfalfa are identical, erect a new genus, Thyrospora, with *T. sarcinaeforme* (Cav.) comb. nov. as the type species. The chief character of Thyrospora is the echinulate or warty conidia.

The investigations of the present writer seem to shed some light upon this nomenclatorial tangle. In connection with laboratory tests of fungicides more than 250,000 spores produced in pure cultures from red clover were examined with the low power of the microscope. Not a single spore with spiny walls was ever seen. Numerous supplementary observations of both stained and unstained spore walls with the high power of the microscope and with oil-immersion objectives have substantiated this conclusion. Spores from lesions on alfalfa leaves invariably appear obviously roughened. Examination of specimens of these fungi on clover and alfalfa from Illinois, which were sent to the writer by Dr. L. R. Tehon, revealed that the spores from the clover leaves are smooth, whereas spores from alfalfa leaves are spiny.

Furthermore, the leaf lesions on the two suscepts are different. Those on alfalfa contain much more yellow than those on red clover, although this may be a suscept response. Single conidial cultures from the two plants grown under identical conditions are distinct, as shown in figure 3. Cultures from red clover are velvety black, with practically no aerial mycelium and no signs whatsoever of perithecial formation. Smooth-walled conidia are produced in great profusion. Cultures from lesions on alfalfa leaves, on the other hand, are very much lighter in color, possess none of the velvety black character, have white fluffy aerial mycelium, with but little dark-colored submerged mycelium, and bear only a few conidia that are spiny. These cultures produce a limited number of black sclerotioid bodies which appear to be perithecial primordia. Incidently,



A

B

FIGURE 3. COMPARISON OF SINGLE SPORE CULTURE OF MACROSPORIUM SARCINAEFORME ISOLATED FROM LEAVES OF (A) RED CLOVER AND (B) ALFALFA

if these could be made to produce mature ascospores, they probably would be those of *Pleospora herbarum*, as described by Gentner (1918). This fungus has been reported from a large number of herbaceous stems. Ultra-violet irradiation, following the method used by Stevens (1928), however, has not induced the perithecia to mature. The writer has obtained single ascospore cultures of what he takes to be *P. herbarum* from overwintered red-clover stems two seasons in succession. These gave cultures identical in every respect with those of Macrosporium from lesions on alfalfa leaves, including the production of perithecial primordia and spiny conidia. Neither in morphological characters nor in cultural reactions does this fungus correspond to *M. sarcinaeforme* as isolated from leaf lesions on red clover. It is probable that the Macrosporium from alfalfa and its possible sexual stage, *P. herbarum*, occurs on alfalfa and red clover, but that *M. sarcinaeforme* from red clover is limited to that suscept in nature.

These observations indicate that Tehon and Daniels (1925) are not justified in including the two pathogenes in the same species, especially if the spiny character of the spore walls is constant. Elliott (1917:467) remarks that " echinulation occurred at all times in all the species studied. In some cases part of the spores from a colony were echinulate while others were smooth." The author has found no evidence of variability in the surface character of the conidia of *M. sarcinaeforme* from red clover. Inasmuch therefore as *M. sarcinaeforme* Cav. was not described with spiny spores, it cannot be incorporated as the type species nor even included in the new genus Thyrospora.

Since the original description undoubtedly refers to a smooth-spored fungus on the leaves of red clover, the name *Macrosporium sarcinaeforme* Cav. is reserved for that species. The purposes of this paper do not warrant a comprehensive discussion of the systematic position of the alfalfa organism aside from saying that it probably is not identical with the one on red clover.

Pathogenicity. Malkoff (1902:284) first attempted to show that *Macrosporium sarcinaeforme* Cav. was pathogenic by inoculating detached leaves in moist chambers with spores. Characteristic spots appeared in from five to seven days, but no reisolations are reported. Bain and Essary three years later (1905:503) say, " The Macrosporium disease appears capable of destroying the clover plant unassisted by any other parasite, though this statement is based only on inspection in the field." Krakover completed Koch's rules of proof for the organism. The fungus has been isolated a number of times by this author and has been grown in pure culture. It was then inoculated onto healthy red-clover plants. The typical spots were produced, and the fungus was reisolated, giving cultures indistinguishable from the original. Spores from these reisolations again produced the disease when inoculated onto other red-clover

plants. There seems to be little doubt of the ability of the fungus to produce the disease.

Life history. The complete life history of the fungus has not been worked out. Evidently the sexual stage is unknown as yet.

Gentner (1918), Krakover (1917: 315), and Milburn (1915:72) report the fungus in the seed. It would appear that the occurrence of the fungus within the seed argues for the possibility that primary cycles sometimes are initiated by rain splashing spores from the seed up on young foliage. Krakover (1917) placed soil from an infected field in pots in the greenhouse. Sterile seed when planted failed to produce infected plants as long as he watered the plants from below; but when he watered them from above with the hose, they immediately became infected, indicating that splashing rain is an important factor. Probably this is the chief inoculating agent, the spores coming either from the diseased seed or the infected soil that constitutes the inoculum.

The effect of some of the factors governing spore germination has been tested by the writer.

In studying the effect of temperature on germination, the spores almost invariably have been allowed to remain in each temperature chamber until all the spores germinable at that temperature grow. This gives a flat curve when the results are expressed graphically, but it indicates to what extent spores may be expected to germinate under given temperature conditions. Although it is desirable to learn the minimum time for maximum germination at each temperature, this has not always been done.

In one experiment conidia were germinated in temperature chambers ranging all the way from 3° to 35° C., with intervals of 6 degrees. Germination after forty-two hours was essentially equal in all the incubators, being from 96 to 99 per cent. The germination at 3° C. was 97.6 per cent and that at 35° C. was 99.2 per cent. Obviously the spores are capable of germinating over a very wide range of temperature.

TABLE 5. EFFECT OF TIME UPON CONIDIAL GERMINATION OF MACROSPORIUM SARCINAEFORME AT 3° AND 25° CENTIGRADE

3° Centigrade			25° Centigrade		
Time elapsed (hours)	Number of spores	Per cent of germination	Time elapsed (hours)	Number of spores	Per cent of germination
2	436	1.4	2	476	7.0
3	441	4.0	3	497	35.9
3½	462	6.1	3½	450	75.6
5	504	9.0	5	465	93.7
6	499	9.8	6	462	95.9
9	472	12.5	7	488	97.3
13	432	42.3	8	485	98.2
24	459	84.3			
26	458	90.6			
42	423	97.7			

Another experiment was laid out the following day to get a time curve for the germination at 3° and 25° C., since it had just been shown that germination was the same at both temperatures. The curve should show a more rapid rise at the higher temperature. That such did prove to be the case is borne out by the data in table 5 and by figure 4.

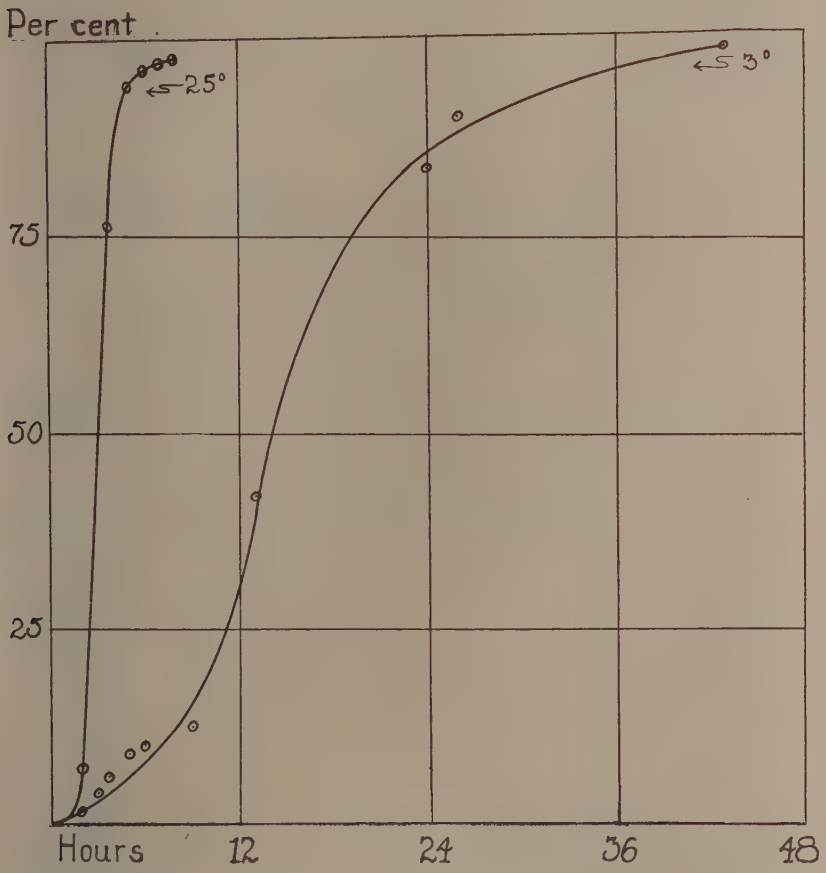


Figure 4. Germination of conidia of Macrosporium sarcinaeforme at 3° and 25° C., showing lag at lower temperature but good final germination

Krakover (1917: 284) by examining the "infection drop" directly under the microscope found that penetration occurs directly through the cuticle after about fourteen hours. Although entrance through stomata occurs, it is uncommon.

At first it was thought that the concentric zones might be cicatrices laid down by the suscept in successive attempts to halt the invasion of the pathogene, but sections through the lesions show, however, neither cicatrice (Cunningham, 1928) nor thickenings of the cell walls which might hinder the advance of the hyphae into adjacent living tissues. In fact, no good explanation of the concentric zones can be offered at this time. The cells within the holonecrotic area are collapsed and browned with disintegrated chloroplasts.

One may assume from the work of Krakover in which he inoculated plants from infested soil that the fungus was living saprophytically in that soil. The vigorous growth of the fungus in artificial culture indicates that it is not unamenable to a saprophytic existence.

Secondary cycles occur throughout the season, and are chiefly responsible for the distinctive development of the disease.

Control

Milburn (1915: 72) upon finding the fungus in the seed recommends seed treatment. To what extent this may be effective and practicable remains to be determined.

Because of the absence of the disease from the experimental areas, two-seasons field experiments with sulfur dust have given no indications of value. On the other hand, a large amount of data regarding the effect of sulfur and copper dusts upon the germination of conidia on glass slides has been accumulated in the laboratory.[2] The effect of the fungicides in inhibiting infection in the greenhouse has been studied as well, but to a less extent.

Laboratory experiments

Effect of sulfur. The results of several experiments on the toxicity of sulfur to spores of *M. sarcinaeforme* are presented in table 6.

This table shows that elemental sulfur in these forms is almost, if not utterly, incapable of inhibiting germination of the spores. In order to see if soluble sulfur, on the other hand, would inhibit germination, a test

[2] The technic for the laboratory testing of fungicides reported in this paper is essentially that long used in the Department of Plant Pathology at Cornell University, and described recently by McCallan (1930). The writer's methods have differed from those of McCallan in a few minor details. Duplicate slides for each treatment were used in each experiment. Each slide contained four separated drops of a spore suspension diluted approximately to 50 spores to each drop, as examined by the low power of the microscope. In determining the percentage of germination, all germinated and ungerminated spores were counted separately in one field of the low power of the microscope. Invariably the materials were dusted on dry slides on which the spore suspensions were placed subsequently. Bank's colloidal sulfur dust is a patented product prepared by fusing finely ground sulfur with bentonite, a colloidal clay, and regrinding. Kolodust is a trade name applied to a dilution of Bank's colloidal sulfur in 300-mesh dusting sulfur.

TABLE 6. Effect of Elemental Sulfur on Conidial Germination of Macrosporium Sarcinaeforme

Experiment no.	Check		Bank's colloidal sulfur dust		Kolodust		200-mesh dusting sulfur	
	Number of spores	Per cent of germination	Number of spores	Per cent of germination	Number of spores	Per cent of germination	Number of spores	Per cent of germination
1	442	99.3	394	93.4	.....	.....	416	97.4
2	411	98.1	409	97.4	397	98.5	412	95.8
3	436	99.6	417	99.3	408	99.5	443	98.8
4	469	100.0	427	97.0	450	99.2	439	99.3
Total or average	1,758	99.2	1,647	96.8	1,255	99.0	1,714	97.8

with N/10 potassium sulfide in comparison with a check and Kolodust was made. The results of this test are given in table 7.

TABLE 7. Effect of Soluble Sulfur on Conidial Germination of Macrosporium Sarcinaeforme

Check		Treatments			
		Kolodust		N/10 potassium sulfide	
Number of spores	Per cent of germination	Number of spores	Per cent of germination	Number of spores	Per cent of germination
211	99.5	212	99.5	201	88.6
225	99.6	196	99.5	170	87.0

The table shows that soluble sulfur reduces the germination about 10 per cent. It appears then that the complete nontoxicity of elemental sulfur shown in table 6 is due in part to its insolubility in the presence of germinating spores rather than to the tolerance of the protoplasm to the sulfur ions exhibited in table 7.

Effect of copper. A large number of tests were performed with copper dusts during the course of the experiments. In table 8 only the results

TABLE 8. Effect of 0.5-Per-Cent Copper-Lime Dust on Conidial Germination of Macrosporium Sarcinaeforme

Experiment no.	Check		Dust	
	Number of spores	Per cent of germination	Number of spores	Per cent of germination
1	419	95.5	376	93.4
2	446	98.5	408	0.3
3	417	98.9	441	58.1
4	487	98.7	442	95.3
5	456	97.1	423	68.5
6	442	97.5	356	48.8
7	417	98.1	465	9.7
Average or total	3,084	97.7	2,911	53.3

with the standard monohydrated copper-lime dust are included. Since in all the tests the 6.8-per-cent metallic copper, diluted with lime, talc, or bentonite, prevented germination entirely until a content of 0.5-per-cent metallic copper was reached, the data in the table refer only to the latter dilution.

The fluctuations of the determinations around the mean of 53.3 indicates that the 0.5-per-cent copper content is at the lower toxic limit.[3] Many factors probably operate to vary the germination from day to day, but the chief one is the variation in the amount of copper on the slides. These tests indicate that a copper dust containing 1 per cent or more of metallic copper should prove effective in field work.

A study was made on the effect of diluents upon the efficiency of the copper lime. In making these tests, one of three substances, lime, talc, or bentonite, was generally used as a diluent for the copper-lime dust. The results obtained from testing these alone for their fungicidal properties are presented in table 9.

The probable error of the differences reveals that the lime and the talc appear slightly toxic, but the differences are so slight that they probably do not have any great significance. Bentonite appears not to be toxic at all.

Greenhouse experiments

A preliminary experiment made in the greenhouse when plants were inoculated after having been sulfured, presaged the results just set forth even before any spores were ever germinated on slides in the presence of fungicides. Plants of mammoth red and medium red clover in pots were selected for inoculation and were divided into two lots, one of which was sulfured with Bank's colloidal sulfur dust and the other was left untreated as a check. The plants were placed in a large moist chamber

[3] One experiment with *Macrospo-ium sarcinaeforme* demonstrated that a dust diluted to a point near what appears to be the minimum of toxicity inhibits germination in varying degrees, depending upon the amount deposited on each slide. Copper-lime dust diluted to 0.5 per cent with talc was applied in heavy and light dosages, as is shown in the following table where the results of the germination tests appear. Except for the check, which was prepared only in duplicate, each treatment was replicated four times each in duplicate, making actually ten tests in all, so that the results should be significant.

EFFECT OF COPPER DOSAGE ON CONIDIAL GERMINATION OF MACROSPORIUM SARCINAEFORME

Treatment	Number of spores	Per cent of germination
Check	487	98.8
Heavy dose	2,161	42.1
Light dose	2,100	98.2

A large number of tests with the same fungus and 1-per-cent copper-lime dust diluted in talc gave no germination, regardless of the thickness of the application. Hence it appears that 0.5 per cent is at or near the minimum point of toxicity. Variations in dosage may be overcome by replication of the experiments.

TABLE 9. Effect of Diluents on Conidial Germination of Macrosporium Sarcinaeforme

Experiment no.	Per cent of germination			
	Check	Lime	Talc	Bentonite
1	98.5	92.1	91.0	....
2	96.0	94.7	67.8	....
3	99.5	96.6	94.5	98.5
4	99.1	96.1	97.6	98.9
5	96.1	94.2	98.6	97.7
6	94.8	97.6	95.2	97.7
7	98.6	81.3	89.0	97.8
8	97.0	86.9	93.5	95.4
9	98.7	97.5	98.6	98.5
10	98.2	99.0	99.1	99.0
11	99.5	97.7	99.5	99.5
12	98.2	98.5	96.9	98.5
13	98.8	98.6	99.1	....
14	98.7	98.6	....	....

Error of the mean*: check, 98.0 ± 0.02; lime, 94.9 ± 0.91; talc, 95 ± 0.07 and bentonite, 98.1 ± 0.01.
Error of the difference: check and lime, 3.05 ± 0.91; check and talc, 2.60 ± 0.07; check and bentonite, 0.14 ± 0.02.

* The probable error of the mean in this bulletin has been calculated according to Bessel's formula:

$$E_m = 0.6745 \sqrt{\frac{\frac{E\,(v-g)^2}{n} - C^2}{n \text{ or } n-1}}$$

for twenty-four hours, and then were transferred to a greenhouse bench until data were taken. The results of two experiments, one conducted in the spring and one in the fall, are given in table 10.

TABLE 10. Effect of Sulfur on Infection by Macrosporium Sarcinaeforme in the Greenhouse

Experiment no.	Pot no.	Check			Sulfured		
		Number of healthy leaves	Number of diseased leaves	Per cent of healthy leaves	Number of healthy leaves	Number of diseased leaves	Per cent of healthy leaves
1	1	188	220	53.9	294	186	38.7
	2	255	50	16.3	205	66	24.4
	3	136	64	32.0	161	48	22.9
	4	119	58	32.7	110	58	34.5
	5	154	160	50.9	187	80	45.2
Average...	...	...	...	39.3	...	...	31.4
2*	1	118	178	39.9	275	221	55.4
	2	103	103	50.0	198	169	53.9
	3	210	152	58.0	96	51	65.3
	4	51	55	48.1	112	145	43.6
	5	79	32	71.1	50	45	52.6
	6	114	112	50.4	108	64	62.8
	7	...	...	....	26	22	54.1
	8	...	...	....	81	45	64.3
	9	...	...	....	16	62	20.5
Average...	...	...	...	45.3 ± 1.86	...	...	42.9 ± 2.4

* E_d in experiment no. $2 = 2.4 \pm 2.62$.

It is seen from the table that on the basis of the percentage of leaves infected, a control of 8 per cent was obtained in experiment no. 1, but the difference is probably not even out of the range of experimental error. The difference due to treatment is much less in experiment no. 2 than in experiment no. 1 and is not significant.

It is evident from table 10 that the experiments in the greenhouse confirm the laboratory tests, showing that *Macrosporium sarcinaeforme* is tolerant of sulfur.

A single test was made in the greenhouse to see if copper-lime dust is as effective in preventing infection as it had been shown to be in inhibiting spore germination. Standard copper-lime dust was applied before inoculation to the leaves that had been moistened previously with tap water. The results of the counts, which were made after eight days had elapsed, appear in table 11.

TABLE 11. EFFECT OF COPPER-LIME DUST ON INFECTION BY MACROSPORIUM SARCINAEFORME IN THE GREENHOUSE

Treatment	Pot no.	Number of leaves	Number of spots	Number of spots per leaf
Undusted checks	1	184	1,065	8.07
	2	230	1,372	8.02
	3	155	631	6.37
	4	178	737	5.94
Average	...	...	....	7.10 ± 0.37
Dusted	5	150	407	4.42
	6	252	507	3.17
	7	229	483.	3.63
	8	126	239	3.09
Average	...	...	....	3.58 ± 0.202

Difference = 3.52 ± 0.42

$\frac{D}{E_d} = 8.38$

Obviously the copper did prevent some infection, because the leaves in the dusted lot averaged only about 50 per cent as much infection as the undusted leaves. The laboratory tests, however, indicated that better control should have been obtained. A single greenhouse experiment is insufficient to warrant the conclusion that copper-lime dust would prove ineffective under field conditions, especially in view of the results obtained in the laboratory.

When all the tests are considered, it appears that sulfur dusts or sprays cannot be relied upon to give control of this disease but that copper-lime dusts are promising.

CLOVER RUSTS

All the rusts of the common clovers are included under the heading *Clover rusts* because of their close relationship. This has been deemed necessary since much confusion in their nomenclature exists through failure to distinguish between the fungi on the various clovers. Emphasis is placed on the rust of red clover, and the points of difference between it and the others are emphasized where necessary.

Suscepts

Arthur (1912:255) regards the rust on red clover as identical with that on crimson clover and on zigzag clover (*Trifolium medium*). He believes also that the rusts on alsike and white clover are identical but are different from that on red clover. The fungus on alsike and white clover also attacks the following plants, according to Arthur: *Trifolium amabile* H. B. K., *Trifolium amphianthum* T. & G., *Trifolium incarnatum* L., and *Trifolium logani* House. He believes that crimson clover harbors both rusts. Davis (1924 a), however, has separated the alsike rust as a distinct disease.

A perusal of the literature reveals only two references to varietal susceptibility. A meager report by Mains from Indiana states that Italian red clover is resistant (Stakman, 1922:243). An anonymous contributor (1923) from the same State says that "two hundred seven selections were made from a large number of plants studied which showed a high resistance or immunity to rust, many of these being resistant to mildew."

The disease

Writers, almost without exception, have referred to these diseases as *clover rust*. Freeman (1905:292) speaks of them as *clover leaf rust*, but since many other parts of the plants besides leaves are attacked, this name is hardly satisfactory. Since each of the three pathogenes are definitely restricted to certain clovers (Davis, 1917), it seems best to combine the common name of the suscept with the term *rust*, to distinguish the three diseases. They therefore are called *red-clover rust*, *alsike rust*, and *white-clover rust*, respectively.

History and range

Red clover probably is rusted wherever it is grown, for it has been reported from 33 States, from 5 Canadian provinces, from 17 countries of Europe, from Asia (Sydow and Sydow, 1910:131–134), and from Australia (McAlpine, 1906:42–44). Although the rusts of alsike and white clover have not been reported as frequently as has that of red clover, they apparently are as nearly ubiquitous.

Economic importance

Usually the rusts of clover have been regarded as of little importance. Most of the records in the *Plant Disease Reporter* read like this, " common but for the most part of only slight importance " (Haskell, 1919:156). It is interesting that, on the contrary, the early references to the disease in America would seem to indicate that it was worse in years gone by than it is now. It is " of great importance " wrote Freeman in 1905 (1905:166). Miss Howell (1890 a:129) stated that it " prevailed to such an extent during the several wet, cool seasons preceding 1890 . . . that it must be regarded as a disease likely to affect seriously, under conditions favorable to its development, an agricultural crop." Pammel (1891:51) in Iowa said that some plants were so severely attacked that the hands became reddened on touching them. Underwood (1888:302) two years earlier estimated that from 5 to 20 per cent of the value of the clover was being destroyed in the vicinity of Syracuse, New York.

According to Lobik (1922), on the other hand, the disease stimulates the plants attacked, so that a heavier production of both green parts and seed results. Such a statement requires more experimental demonstration.

With the exception of that on alsike, the diseases do not now seem to be of much economic importance in New York. Rust can be observed in practically every planting of red clover after midsummer and occasionally as early as June 15, but in no case has severe infection been observed before the first, and most important, cutting was removed. White-clover rust does appear to be destructive to the plants in many lawns and waste places.

Symptomatology

Although the writer has not yet seen the aecial stage on red clover, Hoffer (1917:326) remarks that " the striking feature of the leaves bearing aecia is that they blacken and wilt in a very short time." He believes that for this reason the aecial stage is commonly overlooked. The aecial lesions, which are hyperplastic on white and alsike clovers, kill the affected leaves only after a considerable time. The pycnia appear first as honey-yellow dots on a much swollen lesion on vein, petiole, or stem of the plant. Aecia, which soon appear, are of about the same color. The swellings are often profound, causing the petiole, for example, to swell to several times normal size. It may also bend at this point. When veins are attacked, the leaf blade becomes greatly distorted.

The uredinia are practically alike on all three clovers. They occur, as shown in figure 5, as minute pale cinnamon-brown pustules on any exposed part of the plant, calyces, stipules, stems, petioles, and leaves. They occur on leaves first on the lower surface, and then on the upper. When uredinia are scattered widely over the blade, little or no yellowing

Figure 5. Uredinial sori of Uromyces trifolii fallens on leaves of red clover (A) and of Uromyces trifolii hybridi on leaves of alsike clover (B)

or other suscept response occurs. If they be numerous, however, the leaf sooner or later succumbs, first turning yellow around the spots. Soon the entire blade is involved and finally turns brown and then falls off. The uredinia do not cause evident hypertrophy.

The telia are distinguishable from the uredinia only by their slightly darker brown color

Etiology

Name, history, and classification of the pathogenes. The nomenclature of the various rust fungi on clovers has been so muddled that it is untangled with difficulty. Kern (1911:3–6) gives a good history of the situation up to 1911. He referred the rust on red clover to *Uromyces fallens* (Desm.) Kern, and this disposition seems to be a good one. He was puzzled about the report of aecia by Miss Howell (1890:131) and believed that they arose as a contamination of the white-clover rust. He listed the aecial and pycnial stages as lacking and suggested the possibility that they occurred on Euphorbia. Since the publication of Kern's paper, the aecial stage has been reported independently by Hoffer (1917:325–326), by Davis and Johnson (1917:75), and by Melhus and Diehl (1917:70), proving this rust to be autoecious as are the other clover rusts.

Prior to the paper of Kern, American plant pathologists placed all the clover rusts in one or another species of Uromyces, but referred them chiefly to *Uromyces trifolii*; some ascribing the specific name to Albertini and Schweinitz, some to Winter, and some to Hedwig.

Liro (1906) already in 1906 had pointed out correctly that the red-clover rust should be differentiated from the rusts on white and alsike clover. He placed the forms on alsike and white clover in *Uromyces trifolii-repentis* (Cast.) Liro, retaining that on red clover in *Uromyces trifolii* (Hedw. f.) Lev.

With *Uromyces fallens* as the name for the rust on red clover and *Uromyces trifolii-repentis* for the one on white and alsike clover, the nomenclature of the clover rusts rested until 1924 when Davis (1924a: 203–219) established a new species, *Uromyces hybridi*, of the alsike form on the basis of suscept specificity of the three fungi and on small differences in the aecial and pycnial stages which he found. Arthur (1929:350–351) in his new book on the plant rusts considers the common clover rusts all as *Uromyces trifolii*, consisting of physiologic forms *U. trifolii fallens* on red clover, *U. trifolii hybridi* on alsike clover, and *U. trifolii trifolii-repentis* on white clover.

Pathogenicity. Miss Howell (1890 b:228) by inoculation experiments first formally connected the various spore forms on the clovers. She procured aecia from telial inoculations and uredinia from aecial inoculations. Telia developed later. Liro (1906:11–15) also proved the pathogenicity of these rust fungi by inoculation.

Life history. The general features of the life history of the clover-rust fungi appear to be those of similar autoecious long-cycled rusts. The telia overwinter on debris of diseased plants, where they produce the sporidia in the spring that infect the young leaves and produce aecia. No detailed studies appear to have been made of the phenomena of inoculation, incubation, and infection in the primary cycle in any of the clover rusts.

Aecia of the primary cycle furnish the inoculum for the first secondary cycles, and uredinia serve in the same capacity for the subsequent secondary cycles. Both aeciospores and urediniospores are borne by the wind to new infection courts.

For germination studies with the rust spores, leaves bearing fresh sori were collected in the field. The spores were washed from these leaves into Syracuse watch glasses with a stream of aerated distilled water from a wash bottle, as suggested by Doran (1922), in order to get mature spores and to avoid injuring them. The data on white-clover rust are very inconclusive because of the difficulty in obtaining good germination of the spores used as checks. It is assumed that no valid conclusions can be drawn when the percentage of germination of the checks is less than 90 per cent.

To study the rôle of temperature in germination, duplicate slides of urediniospores were placed at various temperatures in the incubators. The data presented in table 12 are based on an average of several such experiments.

TABLE 12. Effect of Temperature on Urediniospore Germination of Uromyces Trifolii Hybridi and Uromyces Trifolii Fallens

Temperature (degrees C.)	*Uromyces trifolii hybridi*			*Uromyces trifolii fallens*		
	Number of tests	Number of spores	Per cent of germination	Number of tests	Number of spores	Per cent of germination
3	4	825	41.8	4	812	89.2
9	4	893	87.4	5	1,053	94.9
12	1	172	91.2	.....	.....	.....
15	4	838	91.5	4	736	95.7
18	3	667	93.8	.....	.....	.....
21	4	1,159	92.6	2	315	97.4
25	5	2,104	94.8	4	885	93.2
33	5	2,288	15.9	4	865	6.0

It appears that 3° C. is somewhat too low for good germination for both fungi, and that 33° C. is entirely too high. There is, nevertheless, a wide range of temperatures within which these spores readily germinate. Little

is known of infection phenomena. Waters (1928:189) found that the first uredinia appear after about eight or nine days on leaves of alsike clover inoculated in petri dishes. Telia begin to form in nature about October 1. Waters (1928:189) has shown recently that telia may be induced experimentally by three treatments: starvation of the suscept, with the later addition of a rich food supply; sudden change of the suscept from a well-fed condition to a stage of starvation; and continued supply of food, with a gradual dying of the suscept cells and a lowering of metabolism. The latter condition probably exists in nature, for telia of the red- and alsike-clover form have not been seen before the latter part of September or the first of October. Sydow and Sydow (1910:132) state, however, that telia may appear on white clover from May onward. Teliospores are not greatly different morphologically from urediniospores except that they are darker in color and have a slightly thicker wall. They have also one germ pore, while the urediniospores have several arranged equatorially. Teliospores may be borne directly in the same sorus with the urediniospores or in separate sori. The teliospores serve to carry the fungus over the winter.

Epiphytology

Miss Howell (1890a:129) maintains that the clover rusts are most prevalent in cool, wet seasons. If this were true, the fungus should be at its height in the spring rather than in dry midsummer or after as the writer has always found it.

Control

No actual experimental work on the control of clover rusts seems to have been reported in the literature.

Miss Howell (1890a:139) advocates plowing under the second crop and burning over diseased fields in the autumn. These opinions found subsequent expression in the writings of Pammel (1891:55) and of Freeman (1905:292). Miss Howell (1890a:139) said that "the application of fungicides seems impracticable."

Until the work of Kightlinger (1925) most workers inclined to the view that rusts could not be controlled with fungicides. Much recent work has shown (Greaney, 1928) that wheat rusts may be controlled with sulfur dust. The work herein reported appears to be the first dealing with the control of clover rusts with fungicides.

Effect of sulfur. The results obtained with various sulfurs are extensive and seem to be rather conclusive. Data on the white-clover rust are not presented since the spores germinate poorly. The data on the red-clover and alsike-clover forms are given in tables 13 and 14.

TABLE 13. Effect of Sulfur on Urediniospore Germination of Uromyces Trifolii Hybridi

Experiment no.	Check		Bank's colloidal sulfur dust		Kolodust		Undiluted 200-mesh sulfur	
	Number of spores	Per cent of germination	Number of spores	Per cent of germination	Number of spores	Per cent of germination	Number of spores	Per cent of germination
1	873	96.9	867	78.4	.....	.....	749	88.5
2	923	92.6	.....	.....	871	60.7	928	88.9
3	417	97.5	489	27.8	408	42.4	.....	.....
4	429	95.8	567	19.4	537	39.1	.....	.....
5	435	98.8	418	12.5	448	21.0	460	43.0
Total or average	3,077	96.3	2,341	34.5	2,264	40.8	2,137	73.5

TABLE 14. Effect of Sulfur on Urediniospore Germination of Uromyces Trifolii Fallens

Experiment no.	Check		Bank's colloidal sulfur dust		Kolodust		Undiluted 200-mesh sulfur	
	Number of spores	Per cent of germination	Number of spores	Per cent of germination	Number of spores	Per cent of germination	Number of spores	Per cent of germination
1	797	88.1	1,115	3.8	.....	.....	1,100	84.5
2	1,000	92.0	1,023	24.6	.....	.....	1,221	69.9
3	627	91.6	645	56.9	488	75.8	652	74.9
4	413	97.7	371	30.2	330	33.2	366	75.2
5	473	99.5	400	0.0	.....	.....	.....	.....
6	453	96.2	492	2.8	439	22.3	.....	.....
7	515	94.2	495	7.8	445	17.5	462	26.7
8	447	98.2	519	7.0	466	19.7	.....	.....
Total or average	4,725	94.7	5,060	19.0	2,168	33.7	3,801	66.2

The effects of the various sulfurs on the spores are illustrated in figure 6. These drawings, in conjunction with the rather low germinations obtained, indicate that sulfur offers promise for field work in controlling the clover rusts. The reduction in germination caused by any of the dusts is far from complete, yet the tubes are so short and abnormal that many of them would probably never get into the stomates. Another interesting fact brought out by tables 13 and 14 is that the sulfurs reduced the germination more in 1928 (experiments 5 to 8) than in 1927 (experiments 2 to 4). This may be due to the expression of the influence of seasonal differences on the sensitiveness of the urediniospores.

Effect of copper. Copper-lime dust was not tested against the germination of the urediniospores except in 1928. It would be interesting to have data from 1927 to see if the sensitiveness of the spores to this fungicide

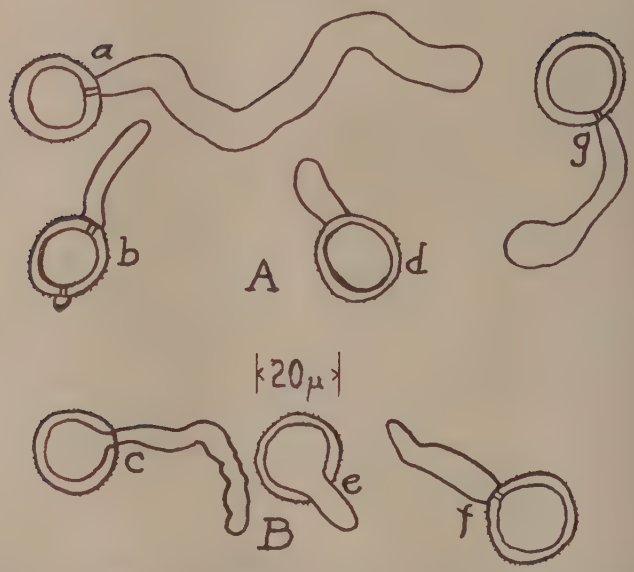


FIGURE 6. GERMINATING UREDINIOSPORES OF CLOVER-RUST FUNGI, UROMYCES TRIFOLII HYBRIDI (A) AND UROMYCES TRIFOLII FALLENS (B), SHOWING THE TOXIC EFFECTS OF SULFUR AND COPPER FUNGICIDES

a, Check; *b* and *c*, in copper-lime dust; *d*, *e*, and *f*, in Bank's colloidal sulfur dust; *g*, in Kolodust

was less in 1927 than in 1928, as was the case with sulfur. The data on this fungicide for both rusts are given in table 15.

TABLE 15. EFFECT OF COPPER-LIME DUST ON UREDINIOSPORE GERMINATION OF UROMYCES TRIFOLII HYBRIDI AND UROMYCES TRIFOLII FALLENS

Uromyces trifolii hybridi					*Uromyces trifolii fallens*				
Experiment no.	Check		7.2-per-cent copper-lime		Experiment no.	Check		7.2-per-cent copper-lime	
	Number of spores	Per cent of germination	Number of spores	Per cent of germination		Number of spores	Per cent of germination	Number of spores	Per cent of germination
1.....	417	97.5	416	33.9	1	473	99.5	439	15.2
2.....	429	95.8	438	35.1	2	453	96.2	411	22.5
3.....	435	98.8	438	33.3	3	515	94.3	447	46.6
	.....	.....	.....	.....	4	447	98.2	469	34.4
Total or average	1,281	97.4	1,292	34.1		1,888	97.0	1,766	29.7

The effect of the copper on the germination is illustrated also in figure 6. A comparison of this figure and the data presented in table 15 with the data in tables 13 and 14 on the effect of sulfur shows that sulfur is more likely to control the disease in the field than is the copper-lime dust.

Effect of lime. A few tests were conducted to see what effect lime alone would have in reducing the germination of the urediniospores. The results are presented in table 16.

TABLE 16. Effect of Lime on Urediniospore Germination of Uromyces Trifolii Hybridi and Uromyces Trifolii Fallens

Experiment no.	*Uromyces trifolii hybridi*			*Uromyces trifolii fallens*		
	Number of tests	Number of spores	Per cent of germination	Number of tests	Number of spores	Per cent of germination
1	2	452	81.9	2	455	97.0
2	2	431	90.8	.....	.....	.....

When these results are compared with the checks, given in table 15, it appears that lime is not toxic to *Uromyces trifolii fallens* and only slightly so, if at all, to *Uromyces trifolii hybridi.*

PSEUDOPEZIZA LEAF SPOT

Suscepts

Many of the species of Trifolium have been listed by Jones (1919:5) as suscepts of Pseudopeziza leaf spot. The writer has collected good material on *Trifolium pratense, T. hybridum, T. repens,* and *T. medium,* and the first three species have been inoculated successfully with a pure culture isolated from *T. pratense.* Inoculations upon *Melilotus alba, Medicago sativa,* and *Vicia villosa* with the same culture were unsuccessful.

Williams (1927) reports that early varieties of red clover suffer more from this disease than do the later-maturing varieties. The less-susceptible ones are Vale of Clwyd (medium-early strain), American medium, and Bohemian. Italian, French, Swiss, and Silesian varieties he found to be the most susceptible.

The disease

This disease is usually referred to merely as *leaf spot.* Since it should be distinguished in some manner from the other leaf spots, the name *Pseudopeziza leaf spot* is used here.

History and range

According to the supplements of the *Plant Disease Reporter* this malady occurs from Vermont to California, and from New Brunswick, Canada, south to Virginia and Tennessee. It is found in practically every clover

field in New York. In Europe, it occurs in Italy (Bivona-Bernardi, 1816), Wales (Stapledon and others, 1922:16), Russia (Jaczewski, 1916), Belgium (Lambotte, 1880:264), England (Massee, 1914), and Germany (Voges, 1909).

Economic importance

Pseudopeziza leaf spot undoubtedly is the most serious leaf disease of red clover in New York, as a reference to the survey data will show. From these data it appears that statements to the effect that no especial damage results (Haskell, 1924:307) are misleading. If New York be considered typical of the northern clover-growing States, then the disease is of great importance. Miss Massee (1914:65) states that the disease is far more prevalent and injurious in England than is generally suspected.

Symptomatology

This disease is easy to recognize in the field. The diagnostic symptom is the fimbriately margined, dark reddish, purple, dark olive, or even almost black spots which show on both leaf surfaces. The fimbriate margin does not occur invariably, but when it does, it is an unmistakable symptom of the disease. Such spots are shown in figure 7, A. Sometimes, especially late in summer, the fimbriate condition is almost wholly lacking. This seems to be due to the dry conditions under which the spots develop. Such spots are illustrated in figure 7, B. The fimbriate spot is most pronounced on *T. pratense* and *T. medium*, but it is usually apparent on *T. hybridum*. This character is much less prominent on *T. repens*. Furthermore, the general color tone of the spots on *T. hybridum* and *T. repens* is lighter than on *T. pratense* or *T. medium*.

In the greenhouse very young infections are much lighter in color than are the older spots observed in the field, matching Prout's brown or mummy brown of Ridgway's color standards (1912). It is practically impossible to diagnose this disease in its early stages in the field because the color of the lesions is not markedly different from that of the other leaf spots. Young spots resulting from artificial inoculation are illustrated in figure 7. The spots may vary from mere specks four or five days after inoculation up to 2 or 3 millimeters in diameter under the wet conditions that often prevail in thick stands of clover in the spring. The disease also appears as small elongated dark streaks on leaf petioles. Pure cultures of the pathogene have been obtained from such lesions.

Cunningham (1928:742) says that the histologic symptoms of this disease on red clover are the same as for those on alfalfa attacked by the congeneric parasite *P. medicaginis*.

> In the holonecrotic area [that is, the dead area in the center of the spot] the palisade cells are almost entirely replaced by the stroma of the fruit body while the cells of the spongy parenchyma are either collapsed or filled with the hyphae. In the plesionecrotic zone [that is, the diseased tissue surrounding the dead area] the epidermal cells may be collapsed. The

A

B

Figure 7. Lesions caused by Pseudopeziza trifolii on leaves of red (A) and alsike (B) clovers
The lesions on the alsike leaves are the result of artificial inoculation

hyphae can frequently be seen in one or two cells beyond this point and occasional threads of hyphae can be seen in the mesophyll cells of this region and may extend to within two or three cells of normal tissue. The cells in this zone have their contents more or less disorganized and an occasional palisade cell is filled with some dense brownish substance, but there is little if any collapsing of cell walls. There is no evidence of cicatrice formation.

The minute apothecium in the center of older spots is another infallible evidence of the disease. After a rain or in moist weather these little fruit bodies push through the epidermis and glisten as minute amber-colored, jelly-like globules, but in drying they shrink and darken so that they are difficult to detect. It is these which make determination simple on the alsike and white clovers.

Etiology

Name, history, and classification of the pathogene. *Pseudopeziza trifolii* (Fr.) Fckl. is the cause of this leaf spot. The organism was described first in 1816 by Bivona-Bernardi as *Ascobolus trifolii* and was cited six years later by both Persoon (1822:342) and Fries (1822:165) under that name. In 1834 Madame Libert called it *Peziza trifoliorum* (1834:324). Boudier changed the name again in 1869 to *Phacidium trifolii* when he revised the Ascobolaceae (1869), but in the same year Fuckel (1869: 290) erected the genus Pseudopeziza, making *P. trifolii* (Biv.-Bernh.) Fckl. the type species. It has remained *P. trifolii* to this day, but the specific name is ascribed to various persons. Frequently, (Biv.-Bernh.) Fckl. is cited. The *h* in the second name is an error apparently first made by Fries (1822:165) and copied by subsequent writers. Persoon (1822: 342-343) omits the *h*. This authority is correct according to the so-called American code which accepts the very first specific name applied. If, however, the International code be followed (Briquet, 1912), the authorities are (Fr.) and Fckl. Oudemans (1921:875) lists the following as synonyms: *Ascobolus trifolii* Biv.-Bern., *Mollisia trifolii* Phill., *Peziza trifoliorum* Lib., *Phacidium trifolii* Lib., *Phacidium trifolii* Boud., and *Trochila trifolii* DeNot.

Much confusion existed for a time through the supposed identity of the forms on clover and alfalfa, but Jones (1919) has shown rather clearly that they are different. In this paper, therefore, the organisms on species of Trifolium are considered to be *P. trifolii* and those on species of Medicago to be *P. Medicaginis*.

Pathogenicity. Jones (1919) was the first person to grow *P. trifolii* in pure culture. The writer has isolated the fungus from red clover by planting pieces of leaves bearing very young lesions in separate tubes of oat agar. Apothecia are formed within a month if a bit of sporulating culture is placed at the top of an oat-agar slant and is allowed to discharge spores over it as pointed out by Jones (1919). Since the fungus grows very slowly at first, it is isolated only with difficulty. Subcultures grow somewhat more rapidly, however; but unless transfers are made frequently,

the fungus tends to cease sporulating. By allowing a pure culture to discharge spores onto plants under a bell jar copious infections have resulted repeatedly on red and alsike clovers, and once a few minute spots showed on white clover. Reisolations were successful from the red and alsike clovers, giving a fungus indistinguishable from the original culture. No reisolation was attempted from the white clover. The results of these inoculation studies agree with those of Jones (1919:22), who inoculated red and alsike clover successfully but who did not use white clover.

Frequently infections are so plentiful on red and alsike clover that the leaves appear peppered with small brown spots, as shown in figure 7. Under such conditions apothecia are not developed.

Life history. Overwintered living leaves in the spring bear apothecia that discharge their ascospores to initiate the primary infections. Apothecia have been known to shoot their spores at least 5 millimeters directly upward to agar in inverted petri dishes. According to the work of Buller (1909), air currents are capable of wafting spores from such a height to the lower leaves. No satisfactory explanation of the distribution from one country to another can be offered as yet. There is no good evidence that the fungus is seed borne.

Jones (1919) has made a careful study of the phenomena of incubation and infection of this fungus in red clover. Germination in the laboratory is not more than 50 per cent, as a rule, even when spores are discharged on plain agar, the best medium for the process. A tube is sent out within twelve hours from any point on the circumference of the spore and passes directly through the cuticle into the leaf. Epidermal and palisade cells resist disintegration until hyphae become very numerous. Germ tubes may enter uncongenial suscepts such as yellow trefoil but cause only a slight yellowing before the germ tubes die. Red- and alsike-clover leaves inoculated at Ithaca in the greenhouse showed very minute brown spots within four days. These enlarged up to 1 millimeter within a fortnight, at which time severely infected leaves were dead.

The secondary cycles follow each other at about three-weeks intervals.

Epiphytology

The disease seems to be favored by wet weather. For example, a thick stand of clover in a field near Kingston, New York, situated in a creek bottom on good soil, was very severely affected in June, 1928. The grower attributed the unusual dying of the lower leaves on his plants to the very wet spring, but an examination showed that *P. trifolii* was attacking the leaves heavily. The spots, which were the largest that the writer had ever seen, contained large amber apothecia that were discharging their ascospores in abundance. Undoubtedly this disease played the chief rôle in killing the leaves. Water is highly essential for the discharge of the ascospores and for their dissemination.

It appears also that the disease is more severe under cool conditions. It is most destructive in New York on the first cutting, but this may be due in part to the more humid conditions prevailing during that stage of growth. Another bit of evidence in favor of the cool-weather hypothesis is that none of the Southern States except Virginia and Tennessee have reported the disease. These two States, especially in the mountainous sections, are cooler than the other Southern States. Jones (1919:18) found that spores are produced more abundantly below 18° C. than above that point. If this condition holds for the field, then the disease should be expected to occur more commonly in the Northern States and even there to be worse in the early months of the growing season.

Control

The control of this important disease is an enticing problem which is still to be solved.

Miss Massee (1914:66–67) in England advocates disinfestation of seed with hydrogen peroxide, since she maintains that the fungus is carried on trash with the seed and even on the seed itself. Jones (1919:35) was unable to prevent the introduction of the fungus into fields by seed treatment.

In view of the evidence above as to the suscept specificity of the Pseudopoziza pathogenes, Miss Massee's (1914) suggestion to clean up weed species of Trifolium and Medicago is of little value.

Sulfur dust for the control of this disease has been tried by the writer both in the field and in the greenhouse. In field experiments in 1926 eight contiguous plots, each 1 rod by 3 rods in size, separated by alleys 1 yard wide, were dusted checkerboard fashion with Kolodust, making four dusted and four undusted plots. The dusting was begun on May 22 and was continued at approximately weekly intervals for six weeks. Pseudopeziza leaf spot was the most serious disease on the clover in the plots, so that weight yields may be taken as a fair index of the control of the disease. At harvest time the green weight of clover from each plot was determined to the nearest one-fourth pound. The average of the dusted plots was 331.3 pounds and that of the undusted plots was 315.8 pounds, a difference of 15.5±7.5 pounds as calculated from pairing the contiguous plots. It is obvious that the difference is not significantly great, and, even if it were, it is too small to be of economic importance.

This conclusion was checked by counting all the clover leaves on three scattered areas 1 foot square in each plot. The leaves were divided into four groups: namely, dead, containing more than 10 spots on each leaf, containing less than 10 spots on each leaf, and disease-free. More than 10,000 leaves were examined.

The data thus obtained showed that the dusted plots had 5.3 per cent

more disease-free leaves than the undusted plots. This difference is statistically significant, but not being great, it is in harmony with the field weighings.

In 1927 the plots were cut down accidentally by a laborer before yield data could be procured, but a careful examination just before this cutting showed no appreciable difference between the dusted and the undusted plots.

Several attempts to test the fungicidal efficiency of various fungicides in the laboratory were ineffectual because of the difficulty of obtaining satisfactory spore germination. Greenhouse experimentation on control likewise is unsatisfactory because: (1) the quantity of leaves available for inoculation during any one experiment is limited, since each pot of clover requires a bell jar in the top of which the fruiting culture is attached; (2) sporulation is erratic in pure culture, making uniform inoculation almost impossible; and (3) a good method of taking data is unknown. Too many pots would be required for significant yield data. Leaf, leaflet, or plant counts are unreliable because of nonuniformity of inoculation.

The indications available from field work show that sulfur will prove ineffective as a fungicide, but copper-lime dust warrants further tests since a congeneric pathogene of the vine, *P. tracheiphila*, is controlled with bordeaux mixture (Faes and Staehelin, 1927).

The work of Williams (1927) indicates that resistant varieties may offer some possibilities for control.

POWDERY MILDEW OF CLOVER

Suscepts

Salmon in his monograph of the Erysiphaceae of the world (1900:178-181) lists 356 species of plants in 157 genera as susceptible to the pathogene of powdery mildew of clover. This extensive list includes the following species of Trifolium and its near relatives, Medicago and Melilotus: *Medicago falcata, M. lupulina, M. sativa; Melilotus alba, M. officinalis; Trifolium agrarium, T. alpestre, T. arvense, T. filiforme, T. hybridum, T. incarnatum, T. involucratum, T. longipes, T. lupinaster, T. medium, T. minus, T. monanthum, T. montanum, T. moranthum, T. pauciflorum, T. pratense, T. procumbens, T. repens, T. rubens.* Salmon records the fungus also on other legumes, among which may be mentioned 12 species of Vicia, 2 of Phaseolus, and 1 of Pisum. The work of Hammarlund (1925) and of others, discussed later, indicates clearly, however, that little danger of cross infection exists, because of physiologic specialization, each suspect species having its own race of the pathogene.

In New York, the writer has seen the disease on alsike, zigzag, red, and white clovers. No powdery mildew has been collected on Medicago or Melilotus. Moreover, a survey of American writings on meadow-crop

diseases has failed to reveal a single record of the fungus on any species of these two genera.

Mains (1923:312) inoculated strains of red clover from many sources in the greenhouse and found that the North American varieties were generally susceptible, while the European showed in general rather high resistance. Another thing noticeable was the presence of resistant individuals in all except three of the varieties tested.

Monteith (1924:63) says, "The Italian clovers which are most susceptible to anthracnose are most resistant to mildew." That European red clovers are resistant to this disease is borne out by the observations of Delwiche (1926) in Minnesota and Noll and Irvin (1926) in Pennsylvania.

The disease

The name *powdery mildew* is so characteristic of the disease that when it is coupled with the name of the suscept, it is sufficiently descriptive. In France the disease is called *le blanc* (Noffray, 1920).

History and range

Sheldon first reported clover powdery mildew in this country in 1908 from West Virginia (Plant Disease Bulletin 6:53. 1922), and it was recorded occasionally up to 1921. Late in 1921, however, an epiphytotic of powdery mildew on red clover appeared in the East. Professor F. C. Stewart in conversation with the writer states that he saw the disease early in September, 1921, in New York. According to Stakman (1922:243), "one of the noteworthy pathological features of the year was the unusual presence of clover powdery mildew." Reports such as the following poured into Washington in 1922: "clover mildew new disease in all sections of State; powdery mildew of clover very prevalent; abundant." Haskell and Wood in summing up clover diseases for 1922 state (1923:250), "during 1922 it [powdery mildew] was much more widely distributed [than in 1921] and occurred all over eastern United States from South to North and in eastern Canada. . . . . Apparently, it did not extend beyond the line of the 100th meridian."

The disease in 1923 appeared to spread westward (Haskell, 1924:302) but was less severe than in 1922. In 1924 there was little change in the situation as a whole (Melchers, 1925:181) except that it was more prevalent in some of the Western States, particularly Oregon, Washington, Idaho, Montana, and Utah. This indicates that the eastern form was spreading.

Haskell (1926) summarizing the situation for 1925 says in effect that the appearance of the disease in the Pacific Northwest in 1924 and its increase in 1925 tend to confirm the theory that a new physiologic from of mildew had been introduced into the United States where it was spreading from east to west. By 1925 (Haskell, 1926:369) the disease had

been reported from all the States of the Union except Wyoming, Nevada' New Mexico, Arizona, and California, and from some of the provinces of Canada. In 1926 Kirby and Archer (1927:198) report it as becoming of less importance, and Haskell (1928:347) says it was of still less importance in 1927. Reports for the country as a whole have not yet been summarized for 1928, but, if New York may be taken as a criterion, the disease is about as prevalent as in 1927.

Salmon (1900:181–182) lists the distribution in full but he does not differentiate between the various suscepts, so that it is impossible from his records to determine how widely clover is affected in the various countries of the world.

Economic importance

Although opinions differ, probably the majority of the plant-disease-survey collaborators tend to hold with Valleau (Plant Disease Bulletin **6**:11. 1922) that "it is questionable whether or not it is causing serious damage." Edgerton (page 12 of the same bulletin) says that "the mildew reduces the tonnage of the clover crop and will cause the hay to be slightly off color and of poorer quality." Anderson (page 13 of the same bulletin) reports that in parts of Illinois "the disease is so bad . . . . that when the mower goes through a field a white cloud arises on all sides."

Possibly those who believe that no damage is incurred have not observed plants in the later stages of the disease. A large number of leaves are killed and many others are severely injured. During a hot day in late August or early September diseased plants partially wilt because, no doubt, of the rapidly transpiring mildew mycelium which draws the water from the leaves. A considerable amount of damage certainly results from the mildew in an average year.

Anderson (Plant Disease Bulletin **6**:13. 1922) in Illinois states that "those who handle the dried hay complain that the dust results in a stinging sensation in the nose and throat," and Tehon (page 13 of the same bulletin) in the same State says, "We have heard that a disease or trouble similar to heaves has occurred in the southern part of Illinois from feeding of mildewed clover to stock." Farmers have inquired whether the diseased hay is injurious to stock. According to Haskell and Wood (1923:251), "No actual cases of stock poisoning from this cause were found and feeding experiments conducted with horses, cattle, sheep and swine at the Tennessee Experiment Station resulted in no harmful effects." Probably, however, horses subject to heaves should not be fed mildewed clover hay unless it is sprinkled to lay the dust.

As with other forage-crop diseases, estimates of loss are guesses at best. Temple and Jehle (Stakman, 1922:243–244) estimated that in Maryland in 1921 the second crop was reduced by 5 per cent and the entire crop by 2 per cent. In isolated fields in New York the loss to the second

crop probably would reach 20 per cent, but for all fields it would not be more than from 1 to 2 per cent in most seasons.

An experiment to be discussed under protection gives some clue as to the damage caused by this disease. The experiment was made in the greenhouse during the mildew period of the 1928 season, and the plants were subjected to no more unnatural conditions than they would have had in the field during the same period. At the end of approximately a month, during which time the plants were continually exposed

FIGURE 8. EFFECT OF POWDERY MILDEW ON RED CLOVER
The plants on the left were kept healthy by dusting; those on the right were diseased

to inoculation, the average green weight of the dusted plants was 1.74 times as much as that of the undusted ones, and their dry weight was 1.53 times as much. These data appear in table 17 (page 52). Apparently the mildew reduced the crop from one-third to one-fourth. The two photographs in figure 8 show the difference between the two series.

Symptomatology

In New York the disease rarely appears prior to the first cutting. It may occur as early as July 20, but in 1926, 1927, and 1928 it has not developed in abundance until after August 15. The first sign of the disease is a barely perceptible patch of white cobwebby growth on the

Figure 9. A leaf of red clover in the early stages of infection by Erysiphe polygoni

upper surface of the leaf (figure 9). These patches increase in size, finally coalesce and give the leaf the superficial appearance of having been sprinkled with flour. When a considerable number of leaves are diseased, the whole field appears white from a distance.

By the time the mycelium has covered the leaf, the tissue begins to show necrotic effects. Dark purple to black irregular spots appear in

severely diseased leaves. These increase in size until the whole blade is dead.

Plants, if diseased when small, are markedly dwarfed. This is evident if mildewed seedlings in the greenhouse are compared with unmildewed ones kept healthy by dusting.

Etiology

Name, history, and classification of the pathogene. Powdery mildew of clover is caused by *Erysiphe polygoni* DC. The perithecial stage has been collected in the western part of the United States but has not yet been found in the East. (Plant Disease Bulletin 6:53. 1922.)

That the pathogene has long been known is indicated by the fact that the causal organism is one of the few fungi named by Linnaeus, who called it *Mucor Erysiphe* (Systema vegetabilium, ed. 13, p. 825. 1774). The present name, *Erysiphe polygoni*, was applied by De Lamarck and De Candolle (Flore Francaise 2:273. 1815). It is of interest historically to note that *Erysiphe polygoni* is one of the organisms that Neger used in 1902 in one of the early demonstrations of physiologic specialization in the Erysiphaceae.

The fungus is variable, but the presence of from four to eight spores in the ascus separates it definitely from *E. cichoracearum* with which it is most easily confused. The latter has regularly only two spores in the ascus. Because of the variability of the species even on the same plant, an extensive synonomy has grown up (Salmon, 1900:174–178). *Erysiphe communis* Grev. and *E. martii* Lév. are the most commonly used synonyms.

Pathogenicity. That strains of *E. polygoni* highly specialized in their pathogenicity occur was early recognized by Neger (1902), who showed, for example, that conidia of *E. polygoni* from *Trifolium incarnatum* would not infect *T. repens* or *Vicia sepium*, although they would infect the species of Trifolium from which they came. Salmon (1903:290) stimulated by this work, sowed conidia from red clover upon various closely related species and concludes "that the Oidium on *Trifolium pratense* is a 'biologic form' specially adapted to this species, and incapable of growing not only on species belonging to other genera of the Leguminosae, but also on numerous species,—if not on all other species—within the genus Trifolium itself." He then predicts that "biologic forms" exist on other species of Trifolium. Seventeen years elapsed before this prediction was substantiated by experimental evidence. Searles (1920:279) found indications of "biologic forms" on *Trifolium minus* and *T. hybridum*. Mains (1923:311) confirms Salmon's form on *T. pratense*. Hammarlund (1925) published a monumental piece of work on the specialization of parasitism of *Erysiphe polygoni* and other species. As a result of his experimentation extending over many years,

he set up 26 "formae speciales" in this species. Those on legumes are f. sp. *medicaginis sativae* on *Medicago sativa* and *M. falcata;* f. sp. *medicaginis lupulinae* on *M. lupulina;* f. sp. *meliloti* on *Melilotus albus, M. altissimus,* and *M. petitpierreanus;* f. sp. *trifolii repentis* on *Trifolium repens;* f. sp. *trifolii pratensis* on *T. pratensis;* f. sp. *trifolii hybridii* on *T. hybridum;* f. sp. *trifolii agrarii* on *T. agrarium;* f. sp. *viciae* on *Vicia sativa* and other species of Vicia; and f. sp. *pisi* on *Pisum sativum.* Hammarlund predicts, furthermore, that even more forms will be found when those on other species of Trifolium are investigated.

Blumer in the same year (1925), although maintaining that the forms on Pisum, Lathyrus, and Trifolium are distinct, is inclined to the view that the Trifolium mildew is a single form with the various suscept species showing varying degrees of susceptibility. Considerable evidence, on the other hand, presented by the recent epiphytotic in the United States suggests that even Hammarlund's f. sp. *trifolii pratensis* may represent a group of specialized races. Haskell and Wood (1924:250) say, "There are good reasons to suppose, particularly on account of the conspicuous character of the mildew, its apparent invasion of new localities, and because of the fact that our native clovers are susceptible, whereas European ones are resistant, that we have to deal with an introduced parasite which has found conditions especially favorable in the United States." Haskell (1926:369) says further that the appearance of the disease in the Pacific Northwest in 1924 and its increase during 1925 tend to confirm the theory that a new physiologic form of mildew has been introduced into the United States where it has been spreading from east to west.

Life history. A rather careful review of the literature has revealed little regarding the mode of overwintering and dissemination. The scarcity of perithecia in the East and the South militates strongly against the probability that perithecia are significant sources of primary inoculum. It is likely that the mycelium lives over winter in or on the protected leaves that remain green throughout the winter months. This possibility is made more plausible by the work of Klika (1922:75) who shows that, contrary to the general belief, the haustoria of *E. polygoni* may pass through the epidermal cells and penetrate into the cells of the palisade and spongy tissue, and even into the vascular bundles. He says that this process occurs chiefly in the older leaves in the autumn. The mildew occurs very plentifully in the autumn so that many infected leaves would be present to go through the winter. If so, then the question is, why does not the disease become general until after the first cutting? Where does the organism live during spring and early summer?

The writer desires at this point to indicate an apparent analogy between powdery mildew of clover and late blight of potato. In New York, the two diseases have appeared almost simultaneously during 1926, 1927,

and 1928. Dr. Charles Chupp in a conversation in the summer of 1928 stated that late blight appeared a little late that year because the nights had been too warm for good germination of the sporangia. Powdery mildew of clover also appeared in epiphytotic form about ten days later in 1928 than in 1927. It appears probable that hold-over mycelium in the overwintered leaves may not be stimulated to form conidia or the conidia may not germinate well except under conditions of cool nights and warm days. It is a common opinion among greenhouse men that powdery mildew of roses is worse where there is a draft, and, according to Salmon (1907), gardeners believe that strawberry powdery mildew becomes more prevalent when a cool period is followed by warm weather.

Some authors (Van Hook, 1906:249; Crawford, 1927:6) maintain that *Erysiphe polygoni* on peas is carried in the seed. This may be true for the clover mildew as well, but no experimental data are available on this point. Infected seed might readily explain the widespread appearance of the apparently new strain of powdery mildew over the United States during 1921 and 1922.

In 1927 an attempt was made to germinate conidia in aerated distilled water in the laboratory; it was a dismal failure, for only a few short or otherwise distorted germ tubes were obtained. The spore material for subsequent experimentation was obtained by washing the conidia from the mildewed leaves into Syracuse watch glasses with a gentle stream of water from a wash bottle, to minimize injury and to get only the most mature spores. The spores were then transferred with a platinum loop to small drops of water on chemically clean slides which in duplicate were placed in moist chambers at room temperature and were left for thirty hours.

In one experiment conidia dusted dry on duplicate slides and placed in moist chambers were compared with conidia in drops of aerated distilled water. A small difference in favor of wet slides was noted, but, even under wet conditions, the germination hardly exceeded 10 per cent.

Since many chemicals are reputed to stimulate the germination of refractory spores, an experiment was laid out to test the effect of these upon the germination of the conidia of *E. polygoni*. The following treatments were used: (1) a quantity of spores were placed in 1-per-cent citric acid and were removed at five-minute intervals; (2) spores were exposed to ether in a moist chamber for ten minutes; and (3) spores were germinated directly in the following solutions: 0.5-per-cent ethyl alcohol, buffer solutions of pH 5 and pH 7.2, clover extract, distilled water with clover leaves in a moist chamber, and distilled water alone. All the moist chambers were held under identical conditions in the laboratory. The germination in most of the solutions, including the buffer solutions, ether, alcohol, monobasic potassium phosphate, and in water (checks), was approximately 5 per cent, the same percentage as in the preceding experiments. The average

germination of the spores in the clover extract on two slides was 19 per cent. Apparently the clover material stimulates the germination. The effect may be due to carbon dioxide acting on the pH of the water, as shown by Platz, Durrell, and Howe (1927) for *Ustilago zeae*, but this was not investigated further since the stimulation was not great.

The conidia may germinate within twelve hours, usually pushing out a germ tube terminally, sometimes laterally. The germ tubes grow slowly in distilled water and show signs of disintegration after about forty-eight hours. The protoplasm becomes vacuolate and hyaline. In clover extract the tubes become somewhat longer and die somewhat less quickly than in distilled water, but none the less surely. The protoplasm remains in the end of the germ tube. This was observed in one case as the protoplasm flowed from the spore to take up this position in the tube.

The first hint of mycelium on the leaves in the greenhouse may be detected within four days after inoculation. The patches of mycelium increase in diameter and fuse, producing within two weeks small necrotic spots which enlarge, finally coalesce, and kill the leaf.

The most marked pathogenic effect of the mildew fungus on the clover plant is stunting. Some data bearing on a possible explanation of this effect are given in table 17. There was a marked difference between the size of the mildewed plants and the unmildewed ones when they were harvested. A glance at table 17 reveals that the disease-free plants averaged 1.73 times heavier than the diseased ones when green and 1.53 times heavier when dry. It appears therefore that the mildew exercises a profound deleterious effect upon the economy of the clover plants. The decrease of the ratio between the two with drying indicates clearly that the diseased plants contain less water than the healthy ones, both upon an absolute and a per-gram-dry-matter basis. This is borne out by the data in table 17 which show that the treated plants average 5.39 grams of water per gram of dry matter and the untreated averaged 4.65 grams. The conclusion obviously is that the untreated plants were living under physiologically dryer conditions than were the treated plants.

Professor H. H. Whetzel in conversation with the writer has suggested that the stunting of mildewed plants is due to the dry condition that has been shown to exist within the tissue and that this dry condition is due to excessive transpiration of the diseased plants. The water is lost rapidly from them through the thick mat of hyphae spread over the leaf. These cylindrical hyphae expose much more surface to the dry air than does the flat leaf surface they cover, even if that were uncuticularized and free of hyphae. They lose water so rapidly that they can hardly be removed from the leaf quickly enough for microscopical examination without shrivelling.

It is well known, of course, that high transpiration causes the following effects: the stomates tend to remain closed, keeping out CO_2; the dry walls

of the substomatal chambers pass but little CO_2 and O_2; water for the production of carbohydrates is scarce; and the process of changing sugars to starch tends to reverse itself. The ultimate effect can be nothing but an undersized, scrawny plant.

The results of this experiment support this transpiration theory, because it was noted several times during the course of the experiment, which ran during hot weather, that the mildewed plants wilted very soon if the gardener was a trifle lax with the watering hose. It appears that they lost water more rapidly than the unmildewed plants.

Conidia produced in the primary cycle constitute the inoculum for the secondary cycles which follow each other at less than two-weeks intervals, because the time from inoculation to spore production and further inoculation usually is not longer than about ten days under summer conditions.

Foex (1924) describes the formation of conidia. He says that the vegetative hypha swells just below a nucleus; the nucleus divides and a septum is laid down. The upper nucleus divides again, and a septum separating these two daughter nuclei is laid down, the upper cell becoming a conidium. The lower cell becomes a spore mother cell from which conidia are cut off in succession. The conidiophores of this species are commonly said to be single-spored, but Salmon (1903:265) points out that chains of from 4 to 8 spores may occur. These chains usually escape detection because the spores separate immediately upon mounting in water.

The factors governing the formation of perithecia would be of interest because of a possible explanation of their absence in the East. The opinion is commonly held as a result of Kleb's work (1900) that starvation, or a lack of nutrients, stimulates reproduction in fungi. Klika (1922:75) maintains that, "Die Entwicklung der Perithezien wird entweder durch das Sinken oder ein Schwanken des Substratnährwertes verursacht." Possibly the report from the State of Washington (Haskell, 1926:369) that perithecia of clover powdery mildew were collected on alsike and white clovers is merely another indication of the same tendency, especially if Blumer's (1925) contention is true that these clovers are resistant to the *trifolii-pratense* form and do not harbor separate forms themselves. Fromme and Wingard (1921:392) found that the uredinial generation was frequently elided in varieties of beans resistant to rust. The telial sori appeared instead.

Epiphytology

As has been indicated under inoculation in the primary cycle, the cool nights and warm days of middle and late summer seem to have a marked effect upon the spread of mildew. Wind plays a significant rôle in the

summer dissemination of the conidia which are very light and easily blown about. To keep clover plants uninfected in the greenhouse in the autumn is difficult because wind-blown conidia filter in through the ventilators and doors. Moisture is of less importance in the development of this disease than in some others because the conidia of *E. polygoni* will germinate on a dry glass slide in a humid atmosphere almost as well as in a drop of water.

Control

Much of the prevailing opinion regarding control of clover powdery mildew is summed up in a statement by Young (1923:160) when he says, "Practically nothing can be done to control clover mildew under field conditions." Mains (1923:308) holds that "control of a disease of the nature of mildew under the cultural conditions necessary for clover is extremely difficult."

Unless the fungus occurs in the seed, as Crawford (1927:6) has indicated for the pea mildew, then exclusion measures would be useless. Crawford obtained control of the primary infections by treating the seed in hot water at 56° C. for twenty minutes. Even in that case secondary infections soon overwhelmed his plants and the disease became as bad as if he had not treated the seed.

The epiphytic nature of powdery mildew renders it highly sensitive to chemicals applied to the plant. In the greenhouse on two occasions severely diseased red-clover plants in pots or flats were divided into two lots, one of which was dusted twice at two-weeks intervals with Kolodust. Within a month the dusted plants were entirely free of infection and showing a dark, healthy green as contrasted with a sickly yellow and brown appearance of the undusted lot.

Sulfur dust has been almost universally recommended for the eradication of powdery mildews of various suscepts. The use of this material in such a way extends back many years into phytopathological history. What appears to be the first attempt at controlling the disease on clover in the field was reported by Barss (1926) in Oregon. He states that two county agents sprayed clover fields with lime sulfur, $1\frac{1}{2}$ gallons in 100 gallons of water for each acre of clover, and found that the mildew disappeared in five days. There was an increase of $2\frac{1}{2}$ bushels of seed to the acre at a cost of about $1 an acre. Fifteen pounds of sulfur dust to the acre also gave satisfactory control. Barss says, "Hot weather is believed to be essential in getting a thorough control with sulfur dust since the heat causes the sulfur to volatilize and give off fumes which kill the fungus and its spores." This is improbable, as is pointed out in the discussion of the Heterosporium leaf spot of timothy, where it is shown that temperature seems to exert little effect upon the fungicidal action of sulfur. It is more

probable that the germinating conidia exercise a solvent action on the sulfur, as suggested by the work of Marsh (1929).

If sulfur dust will eradicate clover mildew once it is established, it certainly should prevent initial infections. An experiment in protecting seedlings was carried out in the following manner in the greenhouse during the mildew period of 1928. Soil of uniform composition was placed in 156 four-inch pots which were planted uniformly to medium red clover on August 9. The plants had become well established by September 6, when the first natural infections were visible. On that date the pots were divided into two groups of 78 pots each. One group was dusted with Kolodust and the other was left untreated as a check. Sixteen pots of heavily mildewed clover plants equally spaced around the experimental pots served, with other mildewed plants about the greenhouse, to insure an ever-ready and a copious supply of inoculum, which eliminated the necessity of applying conidia artificially. A subsequent application of Kolodust on September 12 and another on September 24 kept the treated plants practically free of infection. On October 9 the plants from each pot were cut at the ground line, weighed green, and then weighed again after drying for two weeks at 105°C. The data procured are given in table 17.

TABLE 17. EFFECT OF KOLODUST IN PROTECTING CLOVER AGAINST ERYSIPHE POLYGONI IN THE GREENHOUSE

	Weight in grams, per pot			
	Dusted		Undusted	
	Total	Average	Total	Average
Green plants	1,042.3	13.36	604	7.74
Dry plants	162.83	2.09	106.73	1.37
Water	879.46	11.27	597.26	6.37
Water per gram of dry matter	.....	5.39	.....	4.65

Obviously the sulfur controlled the disease, because the dusted plants averaged 1.73 times as heavy as the undusted when green and 1.53 times as heavy when dry. That means that the dusted gave an average of one and one-half times as much dry hay as did the checks.

Differences in resistance have only been suggested thus far, but crosses between resistant European clovers that are susceptible to anthracnose and winterkilling and American strains that are resistant to the latter two maladies and susceptible to powdery mildew might give desirable plants that would be satisfactory from all points of view. Mains (1923:312) states that individuals resistant to mildew exist even among native clover varieties. Selections from these also should produce desirable strains.

CERCOSPORA LEAF SPOT

Suscepts

The writer (1929) has shown that red, zigzag, alsike, white, sweet, and hop clovers, yellow trefoil, and alfalfa are affected with Cercospora leaf spot in New York. Records of the following additional suscepts are recorded in the literature: *Trifolium alpestre* (Saccardo, 1882:556), *T. incarnatum* (Chester, 1890:96), *T. rubrum* (Passerini, 1877:124), *Medicago arabica* (Ranojevic, 1914:420), *M. denticulata* (Ellis and Everhart, 1891), and *M. hispida* (Wolf, 1916:301).

The disease

History and range

This disease is widely distributed in the United States. It was first reported from New York by Peck in 1883. It is also recorded from New Jersey (Ellis and Everhart, 1888:7), Delaware (Chester, 1890:96), Alabama, Iowa, and Texas (Ellis and Everhart, 1891:89), Mississippi (Tracy and Earle, 1895 b:119), Indiana and Michigan (Stakman, 1922:248), Georgia (Haskell, 1926:366), Missouri and Kansas (Kirby and Archer, 1927:197), Utah (Haskell, 1928), and North Carolina (Wolf and Lehman, 1924). The European distribution is indicated by records from Servia (Ranojevic, 1914:420), Italy (Passerini, 1877:124), Austria (Magnus, 1905:558), and Hungary (Penzes, 1927). There is no record of its occurrence in Asia, Africa, or Australia.

Symptomatology

The lesions produced by Cercospora leaf spot are almost without exception somewhat angular and more or less delimited by the veins of the leaf.

The spots on red clover (figure 10, A) and on hop clover are linear and sharply delimited. Terminal lesions on the leaflets are triangular, being limited on either side by veins from the midrib. Sometimes lesions occur on the pulvini of the leaflets, causing them to yellow and fall.

The elongated spots on red clover are in contrast with the almost circular spots on sweet clover, and almost all intergrading conditions are found on the other suscepts. Lesions on sweet clover may be 5 millimeters in diameter. As a rule, alfalfa bears spots of the same general shape as those on sweet clover but smaller in size. In the spring of 1928 near Cairo, Greene County, New York, secondary shoots of alfalfa developing under moist conditions before the first crop was cut showed elongated lesions precisely like those illustrated on red clover. It appears then that atmospheric conditions and the type of tissue influence the shape of the spot. Alsike, white clover, and yellow trefoil have spots intermediate in shape between the circular ones on sweet clover and the linear ones on red clover.

FIGURE 10. LESIONS CAUSED BY CERCOSPORA ZEBRINA ON LEAVES OF RED CLOVER (A), WHITE CLOVER (B), AND YELLOW TREFOIL (C)

These spots are limited by veins to a large extent and are somewhat elongate but not sufficiently to form definitely linear lesions (figure 10, B and C).

Although the color of the spots varies considerably, the general tone is reddish or smoky brown. Comparisons with Ridgway's (1912) color standards show the lesions to be chocolate or warm sepia on sweet clover; olive brown to wood brown on hop clover; deep brownish drab, light seal brown, and Hay's brown on yellow trefoil; bister, seal brown, bone brown, and clove brown on red clover; Rood's brown and Prout's brown on alsike clover; and burnt umber, warm sepia, chocolate, and bister on white clover. The agreement of color symptoms on many of the suscepts suggests the identity of the disease. Not only the shape but also the color of the spots on white clover is intermediate between that on sweet clover and red clover. Warm sepia and chocolate are common to the spots on white and sweet clovers, while bister is common to the spots on white and red clovers. Light seal brown is common to the spots on yellow trefoil and red clover. No fresh material of diseased alfalfa leaves was available for color comparisons, but here also various shades of brown prevail.

The disease occurs on the stems and petioles as somewhat sunken lesions with colors similar to those of the lesions found on leaves. Such stem infections are serious, since they cause the distal parts to wilt and die.

An ashy-gray down on mature spots on leaves and stems is a diagnostic sign of the disease. This down is composed of multitudes of spores and conidiophores covering the surface of the lesion.

Etiology

Name, history, and classification of the pathogene. The writer has given his reasons elsewhere (1929) for referring the fungus that causes this disease to *Cercospora zebrina* Pass. It is sufficient here to state that material from all the suscepts found in this State and from all the specimens available in herbaria agrees even in minute details with exsiccati type material of this fungus collected by Passerini (1887) himself and distributed by Rabenhorst. In that paper the writer lists the following names as synonyms: *C. davisii* E. & E., *C. helvola* Sacc., *C. medicaginis* E. & E., and *C. stolziana* Magnus. All these names have been applied to fungi on the various suscepts collected from time to time since Passerini first described *C. zebrina*.

Pathogenicity. The fungus has been isolated repeatedly in pure culture from single spores. A photograph of a typical petri-dish culture on oat agar appears as figure 11. Alsike and white clovers have been inoculated successfully with conidia collected in the field and with conidia from pure culture. Although the fungus was not reisolated to fullfill Koch's rules of proof, there seems to be little reasonable doubt of its pathogenicity.

FIGURE 11. A TYPICAL SINGLE SPORE CULTURE OF CERCOSPORA ZEBRINA ON 15 CENTIMETERS OF OAT AGAR

Life history. All the evidence points to seed distribution of the fungus. The author repeatedly has found infections in the greenhouse on primary leaves of alsike and white clover. When seeds from the same lot that produced these infected plants were examined, the superficial mycelium of the fungus, as found by Hopkins (1921) on bur-clover seed, was seen.

Wolf (1916) found conidia in centrifuged washings from seed. Whether these or the seed-borne mycelium or both initiate the primary cycles is

uncertain. In any case, conidia produced on the primary lesions are splashed and blown about to initiate secondary infections at intervals of a fortnight or less.

Figure 12. A conidium of Cercospora zebrina from a leaf of alsike clover, germinating in the presence of Bank's colloidal sulfur dust

No toxic effects are present except possibly a shortening of the tubes

The conidia send forth hyaline germ tubes from one or several cells, as shown in figure 12. Spore-germination studies were limited because conidia ordinarily are not produced in culture. In one experiment, however, spores were washed from an alsike-clover leaf and were germinated in distilled water in duplicate at various temperatures. The data appear in table 18.

TABLE 18. Effect of Temperature on Conidial Germination of Cercospora Zebrina

Temperature (degrees C)	Total number of spores	Per cent of germination
3	302	37.8
9	363	88.6
15	362	92.5
21	360	96.3
26	157*	98.1
33	301	77.7

* Only one test.

The counts were made after twenty-three hours had elapsed, so that a fairly sharp curve resulted. If more time had been allowed, the germination doubtless would have been better at the lower temperatures. An optimum at about 25°C. is indicated. An abundant oxygen supply seems necessary for germination. Some fresh spores accidentally left overnight in a drop of water under a cover glass in a moist chamber were examined the next morning. Spores at the edge of the mount were germinated well, with long tubes, but those in the middle were ungerminated. There was a marked gradient of germination toward the center of the cover glass.

Control

Wolf (1916) and Hopkins (1921) recommend exclusion by seed treatment with formaldehyde, 1 pint to 30 gallons of water.

Two laboratory tests to determine the effect of sulfur and of copper dusts on spore germination were made on spores from alsike and hop clover. The data are given in table 19.

TABLE 19. EFFECT OF COPPER-LIME AND SULFUR DUSTS ON CONIDIAL GERMINATION OF CERCOSPORA ZEBRINA FROM HOP CLOVER AND ALSIKE CLOVER

Treatment	Hop clover		Alsike clover	
	Number of spores	Per cent of germination	Number of spores	Per cent of germination
Check	382	90.2	413	98.6
Bank's colloidal sulfur dust	313	33.9	392	68.9
200-mesh dusting sulfur	...	....	403	96.2
7.2-per-cent copper-lime dust	350	0.0	405	2.0

A camera-lucida drawing of a spore germinating in sulfur is shown in figure 12. A drawing of a spore in 200-mesh dusting sulfur would answer for germination in the checks because it was not different.

It would appear, therefore, that copper dusts are more likely to prove valuable in the field than are sulfur dusts, although Kolodust might give some control because of the colloidal sulfur that it contains.

SOOTY BLOTCH OF CLOVER

Suscepts

In New York all three common clovers are affected with sooty blotch. It probably is most common on alsike but scarcely more so than on white clover. Red clover rarely is affected, although two collections have been made, and in each case the attack was severe. Many other species of Trifolium have been recorded as suscepts. These are as follows: *T. agrarium, T. alpestre, T alpinum, T. elegans, T. filiforme, T. fragiferum, T. lupinaster, T. medium, T. montanum, T. reflexum, T. rubens, and T. wormskioldii.*

The disease

The name *sooty blotch* most adequately characterizes the disease. Cooke (1906:247) speaks of the summer condition, *clover black mould*, and the autumn condition, *clover leaf blotch*. *Sooty spot* is the name commonly applied in the summaries of the *Plant Disease Reporter*.

The disease has been collected in widely separated localities in New York. In the various summaries of the diseases of cereal and forage crops in the United States, this disease has been reported from Minnesota to North Carolina and from Vermont to Washington. Probably it occurs in the United States coextensive with the range of clover. Cooke (1906:247)

lists many European countries and Siberia as being within the range of its distribution, and says that it " is not at all uncommon " in England. Estimates in the records of the *Plant Disease Reporter* vary from " injuring 75 per cent of the crop " in Vermont (Haskell, 1919:156) to " 1 per cent injury was estimated " in Delaware (Johnson and Haskell, 1920:73). It has been reported as " one of the most common leaf spots " in North Carolina (Stakman, 1922). Possibly a quotation from Tehon of Illinois will indicate sufficiently well the probable situation with regard to the disease on alsike clover in New York (Haskell and Wood, 1923:255):

> It is a matter of interest to me to find that in the western part of Illinois . . . . the white clover used largely for pasture purposes is covered with the mold. . . . Practically all pastures where this clover is grown are infested. The infection of plants varies from 30 to 70 per cent and from 60 to 70 per cent of the leaves on these plants are affected with a reduction of photosynthetic surface of 2 to 30 per cent.

Killian (1923) maintains that when white clover is attacked only lightly, the malady is somewhat beneficial because the tissue tends to remain green longer. He assumes, of course, in this case that the green tissues function normally, but this is unproved.

Symptomatology

Both dorsal and ventral aspects of diseased leaves of white clover are shown in figure 13. The first signs are minute black pimples which enlarge more or less circularly on the under leaf surface until a vein is

Figure 13. Lesions caused by Dothidella trifolii on leaves of white clover

reached. They then enlarge somewhat and become angular in shape, since the vein seems to limit the radial growth. At this stage the black blotches appear mealy because of the conidiophores and spores that are present. In the autumn these velvety black, somewhat elevated patches are replaced by other black areas which are shiny above but appear warty if examined closely. The warts are the openings of the pycnidia which develop at this stage.

As the black patches, or stromata, grow, the leaf yellows somewhat immediately about them. This is evident on the dorsal side of the leaf directly above each stroma. A casual glance might confuse this aspect of the malady with clover rust. With increasing secondary infections, the yellowed areas spread, finally causing the entire leaf to die and fall off. As already pointed out, Killian (1923) maintains that sometimes, if the infection is slight, the diseased leaves remain green longer than healthy ones. Bayliss-Elliott and Stansfield (1924:219) in England state that "the leaflets of the infected leaves take an upright position and are thus easily distinguished from healthy leaflets which are extended horizontally."

Etiology

Name, history, and classification of the pathogene. Sooty blotch of clover is caused by *Dothidella trifolii* (Fries) Bayliss-Elliott and Stansfield, according to the International code of botanical nomenclature (Briquet, 1912) which stipulates that the first specific name applied to the perfect stage must be used. If the American code be followed, the specific name must be attributed to Persoon. He used it for the pycnidial stage, which he called *Sphaeria trifolii* in 1801 (1801:30). Many years after Persoon's original description, Kunze and Schmidt described the hyphomycetous stage as *Polythrincium trifolii* in Deutsche Schwaemme, fascicle 5, page 5 n. 121, (Greville, 1826). In 1817 they republished the description (1817:13). Fries (1829:368) attributes *Polythrincium trifolii* to Kunze alone.

Nothing of importance happened to the nomenclature of the fungus until 1849 when Fries (1849:368) designated it *Dothidea trifolii*, based on a supposed perfect stage. He does not give any description. Twenty years later Fuckel (1869:218) placed the fungus in *Phyllachora trifolii*, listing *Polythrincium trifolii* as the conidial synonym and the pycnidial stage described as *Sphaeria trifolii* by Persoon as the spermogonial synonym. He lists *Dothidea trifolii* of Fries as another synonym.

When Saccardo (1883:613) listed the fungus, he designated it *Phyllachora trifolii* (Pers.) Fckl., admitting all the while that the "status ascophorus nondum repertus sed habitus omnino *Phyllachorae*,"— a free translation being that although the ascogenous stage had not yet been discovered, the general appearance places it in Phyllachora.

In 1884, M. C. Cooke says he found a specimen in Berkeley's herbarium (1884:63) bearing elliptical, continuous, hyaline spores, 10 to 12μ by 5μ, in clavate asci. Ellis and Everhart (1892:597) state that all the specimens which they have seen in America are immature or sterile. Clevenger (1905:160) in a short paper on North American Phyllachoras reports the collection of a fertile specimen having oval, hyaline, 8 to 10μ by 5 to 6μ, uniseriate ascospores.

Theissen and Sydow in their monograph of the Dothideales (1915:511) refer the specimen described by Clevenger to *P. umbilicata* n. sp. on the basis of differences in the stroma. They retain *P. trifolii* but as a sterile form.

Killian (1923) first saw and reported mature ascospores of this fungus. He wintered the stromata and obtained perithecia with 2 to 4 large fusiform asci containing 8 hyaline, bicellular spores, 26 by 6μ elongated, and slightly curved. Since the genus Phyllachora has continuous spores, he transferred the clover fungus to Plowrightia of the hyalodidymae of the Dothideales. Killian obtained infection with ascospores. By obtaining infection with the spores he demonstrated that the so-called spermogonia are really pycnidia.

A year later, in 1924, two English workers, Bayliss-Elliott and Stansfield, unaware of Killian's work, overwintered stromata that produced perithecia containing asci with 2-celled hyaline spores measuring 24 to 26μ by 7μ to 8μ. The authors point out, in a footnote at the end of their paper, however, that Dothidella Speg. is the correct generic name since it antedates Plowrightia Sacc. A Latin description therefore is given of *Dothidella trifolii* (1924:227).

In the autumn, Bayliss-Elliott and Stansfield found 1-celled hyaline ascospores in their material. This indicates that the spores which Cooke (1884:63) found in Berkeley's herbarium and those which Clevenger found lack the septum because of immaturity. Probably, therefore, *Phyllachora umbilicata* Theiss. & Sydow is a synonym of *Dothidella trifolii* (Fr.) Bayliss-Elliott and Stansfield.

Only one other report of historical interest need be recorded here, that is, *Polythrincium trifolii* var. *platensis* Speg. on *Trifolium platense* (Spegazzini, 1911:437). The word *platensis* appears to be a typographical error.

Pathogenicity. A great many attempts by the writer to culture *D. trifolii* have been made, using a varying technic that included spore dilution in acidified agar containing oat, potato, or clover decoctions and plantings of tissue that had been sterlized in mercuric chloride or calcium hypochlorite for varying lengths of time. Uniform failure has rewarded these efforts. Killian in France (1923) got similar results. Successful inoculations with conidia from leaves have been obtained in the green-

house. Comparable results have been obtained likewise by Killian (1923) and by Bayliss-Elliott and Stansfield (1924). The two last-named authors, furthermore inoculated plants successfully with pycnospores and ascospores. The fungus undoubtedly is pathogenic, but apparently is an obligate parasite like the rusts.

Life history. According to Killian (1923) the ascospores, matured in perithecia in dead clover leaves in the spring, constitute the inoculum for primary cycles. Conidia from these infections serve as the inoculum for the secondary cycles. Conidia have refused steadfastly to germinate satisfactorily in the laboratory. In two trials no germination whatsoever occurred in aerated distilled water after eighteen hours, but once about 3 per cent germination was obtained in a decoction of clover leaves. Most of the spores sank to the bottom of the drop, although little difference in germination was noted regardless of whether the spores were at the edge or in the center of the drops, indicating that oxygen is not the limiting factor. Germination of conidia in the clover decoction is illustrated in figure 14.

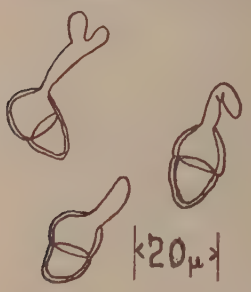


FIGURE 14. CONIDIA OF DOTHIDELLA TRIFOLII EIGHTEEN HOURS AFTER BEING PLACED IN A CLOVER DECOCTION

Note the gnarling of the germ tubes on the spore at the upper left denoting disintegration. Spores placed in distilled water refused to germinate

Killian says that the conidium on the leaf forms a short germ tube which penetrates the cuticle directly at the point of contact with the radial wall between two epidermal cells. Bayliss-Elliott and Stansfield, on the other hand, saw entrance through stomata as well as penetration directly through epidermal cells (1924:221). The mycelium at first invades intercellularly and later intracellularly. Within from six or nine days the black stromata form and push through to the lower leaf surface.

In the autumn conidia cease to form and the stromata containing pycnidia become shiny black and papillate. According to Bayliss-Elliott and Stansfield, as well as Killian, spores from these pycnidia are capable also of initiating infections. It is these stromata which later give rise to perithecia during the course of the winter and thus complete the life cycle of the fungus.

Control

Killian (1923) states that the fungus may live in the soil for five years or more and infect clover crops. If this be true, then attempts at eradication by cultivation or rotation would be in vain. Cooke (1906:247) holds that it would " probably be dispersed by spraying with dilute Bor-

deaux mixture." On the other hand this seems unlikely since the mycelium of the fungus is almost strictly endophytic, and hence out of reach of external toxic agents.

Killian found that if he planted crimson clover in September rather than in July, as is the usual practice in France, the crop derives a certain amount of protection against the pathogene because the more vigorous early planted crop is more susceptible.

Several attempts to determine the value of fungicidal mixtures as protectants were futile because of poor germination or none at all in the checks. Bordeaux mixture, as suggested by Cooke, or its equivalent, copper-lime dust, might have some effect as a protectant.

PSEUDOPLEA LEAF SPOT

Suscepts

In New York alsike, red, and white clovers, yellow trefoil, and alfalfa are affected by Pseudoplea leaf spot. Yellow trefoil is here reported apparently for the first time as a suscept. *Medicago hispida*, *M. maculata* (Miles, 1925:683), and *M. falcata* (Saccardo, 1902:554) are listed also as suscepts. Alsike and white clover are the most likely to be found diseased in this State.

The disease

This malady occurs widely on the three clovers in New York. It has been collected in Schoharie and Tompkins Counties on alfalfa and in the latter county on yellow trefoil as well. According to the records in the *Plant Disease Reporter* and of Jones (1916:299), it occurs in Georgia, Illinois, Kansas, Minnesota, Missouri, Wisconsin, Alabama, Virginia, Iowa, Indiana, and South Dakota. Doubtless it occurs in other States as well. The European countries from which it has been reported are Denmark (Rostrup, 1899), Wales (Stapledon and others, 1922), Italy (Pollacci, 1902), and Austria (Bubak, 1909). Puttemans (1905) collected it in Brazil.

Symptomatology

The first symptom of the disease on any of its suscepts is the occurrence of hundreds of minute black, somewhat sunken flecks on both surfaces of the leaves and on the petioles, giving them the appearance of having been well peppered (figure 15). Stands of white clover along roadsides actually are darkened at times by the millons of spots on the leaf blades. Attention is called especially to the lesions on the petioles.

On white clover the spots enlarge slowly but rarely attain a diameter greater than from 1 to 2 millimeters, at which time the center is gray with a narrow dark reddish brown zone about the margin. On alfalfa the spots

FIGURE 15. LESIONS CAUSED BY PSEUDOPLEA TRIFOLII ON LEAVES OF WHITE CLOVER

may become larger but the colors are about the same. The center of the larger spots tends to crumble away, leaving a ragged edge. Miles (1925: 678) reports lesions on stems, peduncles, calyx, corolla, and even on the seed of *Medicago maculata.*

Leaves may turn yellow and absciss when the lesions become very numerous on the petioles or blades. When this condition obtains to a marked degree in white-clover lawns, the whole stand appears yellow and sickly. Defoliation occurs on all the suscepts.

Etiology

Name, history, and classification of the pathogene. The organism has received several names by different investigators. A very brief review of the history of its nomenclatorial vicissitudes will best serve to present the evidence upon which the writer has based his own decision in the matter. Rostrup (1899) first named it *Sphaerulina trifolii* from white clover in Denmark. Hopkins applied this name to a fungus on red, white, and alsike clovers which he reported for the first time in America in 1923. This fungus was described as having asci bearing eight hyaline, three-septate spores, 30 to 33μ by 12 to 15μ in size. *Pleosphaerulina briosiana*

was described on alfalfa by Pollacci from Italy three years later (1902:51). The Italian fungus was first reported in America by Melchers (1915:536), and Jones (1916) a year later confirmed the determination by comparing it with original material. Von Höhnel (1918:162–163) erected a new genus, Pseudoplea, on *Pleosphaerulina briosiana* because he believed the perithecium to be formed in a unilocular stroma and to have no true ostiole. He describes the ascospores as hyaline and muriform.

Three years later Petrak (1921:28–29) removed Pseudoplea from the Pseudosphaeriales where von Höhnel had placed it and transferred it to the Sphaeriales because he maintained that the perithecium had a true wall and a true ostiole. He went one step further and reduced all these names as follows: *Sphaerulina trifolii* Rostrup (1899), *Pleosphaerulina briosiana* Pollacci (1902), *Pleosphaerulina briosiana* Poll. var. *brasiliensis* Puttem. (1905), and *Pseudoplea briosiana* (Poll) v.H. (1918) to synonyms of *Pseudoplea trifolii* (Rostr.) Petrak.

Petrak very probably is correct in combining the organisms on clover and alfalfa under the same species. The only point of separation has been the hyaline spores in clover and the yellow ones in alfalfa and "the universal absence of a longitudinal cross wall in the spores of the clover organism, and the almost invariable presence of the same in the spores of the alfalfa organism" (Miles, 1925:686). Miller (1925:226), however, shows that the spores of the alfalfa fungus become muriform late in their development only and are hyaline when normally discharged, becoming colored only when the light strikes them. The writer, furthermore, has found muriform spores in single spore cultures of the organism isolated from white clover. It appears therefore that spore color and septation are dependent upon the environment rather than upon any innate properties of the organism itself.

Miles (1925) distinguishes the form on *Medicago maculata* as *Pseudoplea medicaginis*, on insufficient grounds as it appears to the writer. The three characters that he stresses in separating it from *Pseudoplea briosiana* and *Sphaerulina trifolii* are: differences in spore size (page 688); the presence of a more evident and a wider sterile border of the thalli on agar cultures, as well as greater abundance of sclerotial bodies (page 681); and on results of inoculation experiments (page 683). The differences between his spore measurements are so small that a mathematical computation of his figures probably would not show them to be significant. The averages, for example, are: alfalfa organism, 31 by 12μ; clover organism, 34 by 13μ; and the bur-clover organism, 35 by 13.5μ. With regard to his inoculation results, it is sufficient to state that the bur-clover organism infected alfalfa and the three species of Trifolium successfully. Conversely, spores of the white-clover organism produced infections on alfalfa and bur clover. The differences in severity of infection which he reports for the three forms on

the different suscepts would seem to indicate physiologic races of the fungus rather than distinct species.

In the absence of more definite proof to the contrary, therefore, the causal organism is considered to be *Peronospora trifoliorum* (Rostr.) Petr., and *Pseudoplea medicaginis* Miles is added to the list of synonyms.

Pathogenicity. Results of inoculation experiments reported in the literature are variable. Those of Miles (1925:683) just cited were successful. Hopkins also reports successful inoculation experiments with mycelium from pure culture and with ascospores discharged from perithecia on white clover (1923:124-126). Miller (1925:234), however, denies emphatically that the fungus on alfalfa is pathogenic. He says, "It would seem that under no conditions can the germ tube of this fungus enter the live epidermis of the alfalfa plant, not even when the latter has been mechanically injured." He believes that the fungus is a saprophyte following insect wounds or alfalfa white spot. The writer has made no inoculation experiments with this fungus.

Life history. The factors governing ascospore production have been studied but little. Hopkins (1923:121) obtained no mature perithecia on culture media. Jones (1916:500), however, showed that ripe perithecia and spores could be obtained if cultures are held at low temperatures or out-of-doors in a shady place, especially in the spring and the fall.

The author first obtained spores of the organism isolated from white clover following Jones' procedure. Transfers of the same culture produced mature ascospores on potato and oat agar between April 30 and June 13, 1927, but none between June 13 and July 20 when placed on a north window ledge. The spore production was not different in tubes sealed with paraffin or unsealed. Also, transfers to oat agar on October 20 had produced no mature spores by November 30, but on March 26 the tubes were examined again and mature viable spores were found. The temperature, as recorded by a thermograph beside the tube, once fell to 5° F.

It appears that spores may be produced in culture in spring or winter, but not under the summer conditions of these experiments. A large number of transfers to the same oat agar were made on March 26, 1928. Half of these were covered carefully with black paper to exclude light and then some covered and some uncovered ones were placed in the greenhouse and outside on the same window ledge as was used the previous year. By May 16 the uncovered tubes on the window ledge contained mature ascospores, some of which were uniform. Not a single spore was seen in the tubes from which the light was excluded. On the same date, however, no spores were present in any of the tubes in the greenhouse where the temperatures had been consistently higher. It appears from these experiments that light stimulates spore production in the presence of low temperatures. The nonproduction of spores in midsummer probably is

due to the higher temperatures. At any rate, it cannot be due to differences in length of day, because in the last experiment described the length of day was the same in the greenhouse as on the window ledge. Of course, the direct sunlight on the tubes in the greenhouse may have inhibited spore production, although it did not inhibit the growth of the fungus.

It should be mentioned incidentally that the black color commonly found in cultures of the fungus was practically absent from the fungus in the tubes from which the light had been excluded. This agrees with Miller's observation that light causes the darkening of ejected ascospores (1925:226).

Hopkins (1923:125) found perithecia on the dead overwintered leaves in the spring which served as sources of inoculum. Miles (1925:678) finding incipient perithecia on the seed believes that this explains distribution. Miller (1925:226–230) germinated spores after they had lain a year under dry conditions. He found that the asci absorbed water, became inflated, burst the neck from the perithecium, and discharged spores as high as 10 centimeters toward the light. Spores germinate within twelve hours and produce several tubes. These, according to Hopkins, penetrate the cuticle directly (1923:126).

Control

Experiments with fungicides in the laboratory were limited because of a paucity of spore material. Spore suspensions usually were too dilute to be of great value even when the cultures could be induced to form spores at all. The results of one experiment, however, appear in table 20.

TABLE 20. Effect of Sulfur and Copper Dusts on Germination of Ascospores of Pseudoplea Trifolii

Treatment	Total number of spores	Per cent of germination
Check	272	93.4
7.2-per-cent copper-lime dust	215	0.0
3.6-per-cent copper-lime dust	80	87.5
Kolodust	88	93.0
200-mesh dusting sulfur	62	98.3
Bank's colloidal sulfur dust	56	96.5

The above experiment illustrates the general reaction of the fungus to copper and sulfur dusts. The spores used came from pure cultures. The fungus apparently is not sensitive to sulfur, so that further work with this protectant looking to field control is likely to be fruitless. Copper-lime dust, however, might give control under field conditions.

CLOVER MOSAIC

Mosaic of clovers is a common virus disease to which little attention as yet has been given by plant pathologists. It undoubtedly deserves careful investigation.

Suscepts

The disease occurs commonly in New York on red, alsike, white, and sweet clovers. It has been reported elsewhere on other suscepts: by Orton on *Trifolium incarnatum* (Fromme, 1921:171); by Mains (1928:363) on *T. arvense, T. resupinatum;* by Elliott (1921:148) on *Medicago falcata* and *Vicia faba;* and by Dickson (1922 a:42) on *Medicago lupulina.* Böning (1927) was able to transmit the disease to beans. It is interesting in this connection that Elliott (1921) was unable to get infection on white clover or alfalfa in Arkansas, whereas the virus from sweet clover and red clover easily cross-infected. Dickson (1922 a:42) had no success in transmitting red-clover mosaic to sweet clover in Canada, but white clover readily became diseased with the same inoculum. Dickson (1922 b:88) says, "In one instance there appeared to be a successful inoculation of *Medicago sativa* by means of aphids." In one trial also he succeeded in inoculating *Trifolium hybridum* with juice from *Pisum sativum.*

Mains says (Kirby and Archer, 1927:199) that no varieties of clover are immune to mosaic in Indiana.

The disease

History and range

The earliest reference to a clover mosaic which the writer has found is that by Clinton (1915:360) who said, "Not infrequently the common species of clover show yellowish-green irregular areas." McLarty (1920: 501) asserts that H. T. Güssow saw sweet-clover mosaic as early as 1912 at St. Catherines, Ontario. Several papers appeared in 1920 and 1921 reporting independent observations on the disease (McLarty, 1920; Orton: Fromme, 1921:171; and Elliott, 1921). Dickson published some observations on plants affected and on the symptomatology of the disease in 1922.

Clover mosaic has been reported from Ontario (McLarty, 1920:501) from Quebec (Dickson, 1922 b); from Germany (Böning, 1927); and in the United States from New York west to Montana and south to Louisiana.

Economic importance

Clover mosaic apparently is not a serious disease in this State, although it occurs in most fields in small quantities. In isolated fields it may be really serious in years when plant lice are numerous. The loss comes from the stunting of plants and the reduction in the amount of seed produced

as well as from the poor stands that result from low germination of seed from diseased plants (Dickson and McRostie, 1922:42). McLarty (1920: 503) says of sweet-clover mosaic that " when growing wild, diseased plants yield about one-half the herbage of their healthy neighbors." In Pennsylvania, C. R. Orton found a six-and-one-half-acre field of crimson clover in which 50 per cent of the plants were diseased (Johnson and Haskell, 1920). Mains (Kirby and Archer, 1927:199) reports a 0.5 per cent loss for Indiana in 1926.

Symptomatology

To any one who is acquainted with mosaic on other plants, the symptoms of this disease on clover will be familiar. The usual mottled, crinkled, and curled leaves are very evident, as illustrated in figure 16. Frequently

Figure 16. Mottling and wrinkling of leaves of red clover results from infection by mosaic virus

the size of the leaf is more reduced than that shown in the photograph. The crinkling is due to an unequal rate of growth between the diseased and healthy tissues, as pointed out by Dickson (1922 b:14). Another hypoplastic symptom is shortening of internodes which, with the dwarfing of the leaves, may at times reduce the size of the plant a half. The inflorescence also is reduced considerably with attendant small yields of seed.

Dickson (1922 b:45) states that, " Leaf thickness in [diseased] red clover varies from 105 microns in the lighter to 150–180 in the darker. . . . . Chloroplasts are fewer and paler in the lighter areas and the intercellular

spaces are reduced somewhat by the muriform cell arrangement. In crimson and red clovers the contents of severely infected cells tend to disorganize."

Etiology

Since the causal agents of mosaic diseases remain obscure, a discussion of this feature of clover mosaic does not come within the scope of this paper.

Whatever the nature of the causal agent of clover mosaic, pathogenic effects certainly are produced and the agency is transferable from one clover plant to another. McLarty (1920:503) obtained infection with juice from diseased plants by inoculating it into healthy leaves both before and after passing it through a Chamberland filter. Dickson (1922 a:42) transmitted the disease by means of aphids.

Dickson and McRostie (1922:42) conclude from their experiments on planting seed from diseased red-clover plants that the malady is seed borne and impairs the germination. Seedlings from infected seed or overwintered infected plants serve as sources of primary inoculum. The agents of inoculation in nature are various sucking insects, among which the aphid *Macrosiphum pisi* Kalt. has been shown to be responsible (Dickson and McRostie, 1922:42). Elliott (1921:147) showed that symptoms appear within from ten to fifteen days after inoculation.

According to Johnson (1922), "clover mosaic is seemingly favored by low temperatures."

Control

But little can be offered yet in the way of control. Selection of seed from disease-free fields should be of some value, but obviously roguing diseased plants as practiced for other mosaic diseases is impractical under present conditions of clover-seed production.

KABATIELLA ANTHRACNOSE OF CLOVER

Except for observations on distribution and severity of the disease, the writer has made but little study of Kabatiella anthracnose of clover. The malady frequently is of grave importance in the State. The Colletotrichum anthracnose has been reported from New York but was never collected by the writer.

Suscepts

Red clover only is commonly affected by this disease in New York. The following plants have been reported elsewhere as suscepts: *Trifolium purpureum, T. medium* (Malkoff, 1902:283); *T. hybridum* (Fulton, 1910: 752), *T. suaveolens, T. repens* (Stapledon and others, 1922:87); *T. in-*

carnatum, *Medicago lupulina*, and *Onobrychis sativa* (Wellensiek, 1926:297). *T. repens* and *T. hybridum*, as well as alfalfa, seem highly resistant if not immune.

In Wales it appears that the late-flowering strains of red clover are less susceptible than are the early flowering ones (Stapledon and others, 1922:85–86). European opinion rates the American clovers high in susceptibility, as indicated in the paper by Westerdijk (1917), whereas in America, the European clovers are said to be more susceptible (Monteith, 1924:63). This point is considered again under the discussion of the distribution of the disease.

Ware, in England, found that Hersnap, a Danish strain, is very resistant (1923:835). Hesler (1925:92–93) points out that the density of stand and the amount of anthracnose vary directly with each other. Tennessee-anthracnose-resistant is reported as susceptible to Kabatiella anthracnose in Kentucky (Haskell, 1928:347).

The disease

Kabatiella anthracnose of clover is an expression logically applied in English because some means of separating the diseases produced by several similar organisms must be devised. It is called "Stengelbrenner" in Germany (Mehner, 1901), "Klaverstengelbrandziekte" in Holland (Wellensiek, 1926), and "scorch" in England (Ware, 1923).

History and range

The first undisputed records of the disease are those of Mehner (1901) and Kirchner (1902) in Germany. According to Stevens and Hall (1921: 331) the disease was first reported in America by Sheldon in 1906 in West Virginia. Stewart (1919:173), however, states that he collected specimens as early as 1903 in New York, The most recent papers dealing with the disease are those of Wellensiek (1926) and Sampson (1928).

Kabatiella anthracnose has a wide distribution. In Europe it occurs in Germany and Bohemia (Linhart, 1902), Switzerland (Stebler and others, 1917), East Prussia (Anonymous, 1926), Bulgaria (Baudyš, 1924), Wales (Stapledon, 1920:637), and Holland (Westerdijk, 1917). It has been reported also from Morocco in Africa (Miège, 1921:39).

The disease occurs generally in New York wherever clover is grown, having been seen in all the counties in which the crop was examined. According to supplements of the *Plant Disease Reporter*, Kabatiella anthracnose has been collected in the United States from Massachusetts to Minnesota and south to Delaware and Missouri. It appears significant that the disease has been collected rarely except in the higher latitudes. The facts pointed out under varietal susceptibility that the southern European varieties seem to be the most susceptible while the northern

ones are resistant may be correlated with this. It is also of interest that the Danish strain, Hersnap, was the most resistant in Ware's trials (1923) in England and that the Tennessee-anthracnose-resistant strain was not resistant to this disease in Kentucky (Haskell, 1928:347). If we postulate that this disease is a northern one, then the clovers grown constantly in its presence are more likely to be resistant, whereas clovers not normally subjected to it should be more highly susceptible. A possible explanation for the northern distribution is set forth by Monteith who shows that the causal organism has a relatively low optimum temperature (20° C) for growth, while the minimum temperature is 4° C. (1926:71).

Economic importance

In the three seasons, 1926, 1927, 1928, Kabatiella anthracnose undoubtedly has been the most destructive disease of red clover in New York.

The anthracnose disease is unlike leaf spots in the character of its effects on the suscept. The disease not only kills the tissues actually invaded, but also affects the tissue beyond the infected area as well and frequently kills it. A lesion of a given size on the stem or the petiole will be responsible for much more injury than a lesion of the same unit size on the lamina of a leaf. For this reason the index of infection for this disease obtained in the survey of first-cutting clover in 1928 indicated the disease to be of less importance than either Pseudopeziza leaf spot or Macrosporium leaf spot, whereas it probably is more important than either.

The index of infection in New York in June 1928 was $\frac{(1)\ (9)\ (33)\ (15)\ (42)}{119}$, which means that 7.92 per cent of the stems and petioles of red clover in the State were affected at that time. Inasmuch as the effect of this amount of necrosis on the general economy of the plant is unknown, no estimate of absolute loss can be given. It may be less than 7.92 per cent or more — probably the latter. Estimates in the literature concerning losses are frequent, but they probably mean little except by way of indicating the relative significance of the disease.

Some workers have claimed that the reduction in yield is very large. Lobik (1915), for example, in his study of the effect of fungi upon clover yields reports a reduction of about 50 per cent because of this anthracnose.

Symptomatology

The most diagnostic characteristic of this disease, as pointed out by Kirchner (1902) in his original description of it, is the occurrence of elongated or somewhat stripe-like, sunken, light- to dark-brown spots surrounded by a broad black zone. Sometimes the latter is not prominent, however. These spots are at first small and somewhat watersoaked,

but they subsequently enlarge in the direction of the long axis of the stem and become sunken so that at times the pith may be exposed. They may reach from 4 to 5 centimeters by ½ centimeter in size. At such stages the upper part of the stem wilts, turns brown, and dies, giving the field a scorched appearance. Infections on tender petioles or flower pedicles causes these organs to wither and fall over. This also is very characteristic of the disease, especially in the spring when environmental conditions are right. Sometimes the pulvini of the leaves are infected as pointed out by Miss Sampson (Stapledon and others, 1922:83-87), but infections of the lamina are rare. In wet weather the minute white fruiting bodies of the fungus, the acervuli, may be seen with a hand lens in the center of the lesions.

Etiology

Name, history, and classification of the pathogene. Four pathogenes, said to cause clover anthracnoses, have been described. Peck (1880:26) described *Gloeosporium trifolii* in New York as occuring on leaves of red clover. Kirchner (1902:12) twenty-two years later described a species *Gloeosporium caulivorum*, which he distinguished from *G. trifolii* because it occurs exclusively on stems and petioles and because the spores are curved instead of straight. He did not detect any differences in size. Very frequently these two have been considered identical in Europe (Baudyš, 1924, and Voglino, 1909). In America, however, *G. caulivorum* Kirch, usually has been cited as the cause of clover anthracnose.

Bain and Essary (1906 a:193) found still another organism, which they named *Colletotrichum trifolii* because the spores are straight and setae are present in the acervuli. This organism, which seems actually different, has been treated thoroughly in the recent publication of Monteith (1928). O'Gara (1915) distinguishes as new *Colletotrichum destructivum* on *T. pratense* and *T. hybridum* because there are no definite spots, and the spores and setae are much larger than those of *C. trifolii* Bain and Essary.

According to a recent paper by Miss Sampson (1928:104), Karakulin (1923) changed *Gloeosporium caulivorum* Kirch. to *Kabatiella caulivora* (Kirch.) Karak. Miss Sampson states, "My own observations show that it is not a typical Gloeosporium, since it is characterized by a scanty growth of mycelium in the host and on culture media, the absence of a definite stroma beneath the acervulus, and the short club-shaped conidiophore bearing a terminal crown of conidia. These characters agree with those of the genus, Kabatiella Bub., as emended by Karakulin."

The causal agent of clover anthracnose in New York agrees with this description, and it is considered here as being *K. caulivora*. It is sufficient to point out with Baudyš (1924) that the position of the fructification is small ground for separating species, and hence that *G. trifolii* Peck. is

probably synonymous with *K. caulivora*. If this should prove to be true, then the name would have to be *K. trifolii* (Peck) Karak. emend. The writer has not seen Peck's specimen.

Pathogenicity. The researches of Mehner (1901:195), Fulton (1910), Wellensiek (1926), and Sampson (1928) have shown beyond reasonable doubt that the fungus is pathogenic to clover plants.

Life history. There has been much controversy regarding the source of inoculum in newly sown fields. The common assumption dating from the paper of Mehner (1901) has been that the fungus is transported with the seed. Fulton (1913:249) says, "The spores when dry retain their vitality for a number of months, and it is likely that such dust-like spores lodged on the outside of seeds may serve to carry infection. Clover trash may harbor the fungus, and serve the same purpose." The point seems still to be in doubt, for Wellensiek (1926:297) says, "As far as exact experiments were undertaken, no carrying by the seed could be observed." Miss Sampson (1928:127) got infected plants by planting inoculated seed in sterile soil under bell jars even though "positive evidence for the transmission of the disease by naturally contaminated seed has not been obtained."

It seems probable from the writer's field observations of anthracnose lesions in the early spring that the fungus overwinters in the perennial green stems. Miss Sampson found a similar condition. Splashing rain undoubtedly is necessary for dislodging the gummy conidia from their acervuli and transporting them to the infection court.

Conidia germinate very rapidly in distilled water, sending out long germ tubes. Miss Sampson (1928:119) placed conidia on succulent stems and studied penetration by stripping off bits of the epidermis at intervals. The cells are penetrated after the third day, but she did not see the actual penetration tube. She assumes that it passes directly through the cuticle. She says that the first evidence of infection is darkening of the epidermal cell immediately beneath the spore. The protoplasm contracts and becomes discolored. Within a week the dark-brown spots appear on the stems on which spores are produced again.

According to Wellensiek (1926:296), "Infection is favored by a temperature of about 20°C. and by great moisture. Drought, on the other hand, seems to make the plants more susceptible."

Control

Many writers, assuming that the organism is carried with the seed, have recommended seed treatment. Baudyš (1924) applied 1-per-cent corrosive sublimate for ten to twenty minutes, but the control was unsatisfactory. Fulton (1913:249) recommends the use of seed from anthracnose-free

fields, or seed that has been cleaned mechanically, and disinfection of the seed with formalin solution, 1 ounce to 3 gallons of water, for fifteen minutes. Hegyi (1915) recommends treating the seed with 1-per-cent copper-sulfate solution.

Unfortunately, no data on the sensitiveness of this fungus to fungicides have been obtained because the spores are so small that they are distinguished with great difficulty from the dust particles on slides. This fact makes fungicidal tests in the laboratory difficult and unsatisfactory. It may be said, however, that in the two seasons of field dusting with sulfur no control of anthracnose was apparent in the plots. Apparently this fungus, like *Glomerella rufomaculans* and other anthracnose fungi, is tolerant of sulfur.

The production of resistant varieties is commonly assumed to be the panacea for control of this malady, but no good ones as yet have been discovered. Miss Sampson (1928:111) advances some evidence to show that much of the apparent resistance which has been observed is in reality only klenducity, because propagants that she took from field-resistant and field-susceptible plants were all affected alike when placed under optimum conditions for infection.

STAGONOSPORA LEAF SPOT

The disease for which the name *Stagonospora leaf spot* is proposed, is widespread in this State, occurring most plentifully on alsike clover. It occurs in New York on white, red, alsike, and sweet clovers, as well as on yellow trefoil and alfalfa. It has not been collected as yet on hop clover. Because of a confusion of names of the causal organism, records of the disease are difficult to identify in the literature, but it undoubtedly occurs on other suscepts also.

The lesions, which vary considerably in diameter, are circular or oval to irregular in shape. Diagnosis is made easy by the light-brown to white center and the dark-brown margin of the spots. Comparisons with Ridgway's (1912) color standards give: for the center of the spot, deep olive buff to pallid brownish drab on white clover, white to tawny olive on sweet clover, pale smoke gray on alsike clover; for the margin of the spot, buffy brown on white clover, bister on sweet clover, and brownish drab on alsike clover. In some instances the spots tend to show faint concentric zones of the same color as the margin. These have been observed on all suscepts except sweet clover. This tends to confuse the spot with the Macrosporium leaf spot, but the signs described in the following paragraphs and the papery aspect of the center of the older lesions differentiates the lesions in such cases.

Greenhouse inoculations with single spore cultures isolated from alfalfa, as shown in figure 17, A, result in spots on alfalfa seedlings that are

hydrotic at first but later dry and show the dark zones and the dark margins evident in the photograph. The papery character of the central portion of the spots on sweet-clover leaves is shown in figure 17.

The minute dark pimples, the pycnidia, are to be seen prominently in the light-colored centers of the spots. These are characteristic signs of the disease.

The author experienced difficulty at first in identifying the causal fungus. It has been classified under various names according to the age of the spores and the suscept involved. The writer at first identified it on red, alsike, and white clovers as *Phyllosticta trifolii;* on yellow trefoil as *Phyllosticta* sp.; on red clover as *Septoria compta* or *Septoria trifolii;* on alfalfa as *Ascochyta imperfecta, A. medicaginis,* or *Stagonospora carpathica;* on sweet clover as *Ascochyta meliloti, A. caulicola,* or *Stagonospora meliloti.* Single spore cultures from the Phyllosticta on alsike and white clovers, the Ascochyta on alfalfa, and the Stagonospora from sweet clover, were identical in appearance under the same cultural conditions. The color of the thalli, the yellow color imparted to the medium (oat agar), and the size, shape, and septation of the spores were the same. The fungi agree in every respect with *Stagonospora meliloti* rom sweet clover. At the completion of this experiment the writer realized the then very obvious correspondence of the symptomatology on the various suscepts and concluded that the Sphaeroidaceous fungi under consideration on the legumes are probably identical. Subsequently in perusing the literature on *Sphaeru ina trifolii,* a paper by Petrak (1919) was discovered in which he has combined all these fungi under the name *Stagonospora meliloti* (Lasch) Petrak. He lists the following names as synonyms: *Sphaeria meliloti* Lasch, *Septoria medicaginis* Desm. et Rob., *Septoria compta* Sacc., *Septoria meliloti* Sacc., *Phleospora trifolii* Cav., *Stagonospora carpathica* Bäumler, *Stagonospora trifolii* Fautr., *Stagonospora trifolii* Ell. & Ev., *Stagonospora dearnessii* Sacc., *Stagonospora medicaginis* v. Höh., *Stagonospora compta* Died., and *Ascochyta caulicola* Laub. This group of synonyms may well include *Septoria trifolii* Ell. & Ev., *Septoria trifolii* Cav., *Ascochyta medicaginis* Bres., *Gloeosporium meliloti* Trelease, *Ascochyta meliloti* (Trel.) Davis, and *Ascochyta lethalis* Ell. & Barth.

Ascochyta imperfecta Peck on alfalfa is certainly different from this fungus which occurs also on alfalfa. The former produces dark purple spots which later become indefinite in outline and yellowed as contrasted with the light-colored spots with a dark margin. Single spore cultures of *A. imperfec a* differ from those of *Stagonospora meliloti* in being almost black on oat agar instead of yellow. Sprague (1929:929) agrees that the two fungi are different, for he says that "there can be little doubt that *A. imperfecta* is different from *A. medicaginis* as shown by inoculation work, coupled with study of material." Apparently Sprague did not

Figure 17. Lesions caused by Stagonospora meliloti on leaves of alfalfa (A) and sweet clover (B)

collect a fungus similar to *S. meliloti* on alfalfa, for he makes no mention of it.

Little data on the life history or the control of this disease are available. It can be stated that single spore cultures of the fungi from alsike clover and alfalfa have been inoculated successfully onto their respective original suscepts; the disease has been produced in its typical condition; and the pathogenes have been reisolated again. No cross inoculations have been made.

GIRDLE

A peculiar girdle disease of alfalfa, red clover, and sweet clover has been found in the State. It appears to be identical with that described and figured by Brown and Gibson (1922) and apparently has not since been reported outside of Arizona, where it originally was found. The writer found it, however, in August, 1929, near Little Rock, Arkansas, on alfalfa (Plant Disease Reporter 13:129. 1929). Diseased plants of all the suscepts have the characteristic symptoms of girdle. Leaves on affected stems first turn purplish, later reddish, and finally yellow in color. Stems bearing such leaves are girdled from one to several times by bands of gray-green tissue, bordered on either side by narrow strips of darker colored tissue. Frequently sarcody, or swelling, occurs above the girdle and adventitious roots may begin to grow from the tumid tissues. Apparently no chewing insect is responsible for the injury, because the epidermis over the girdle remains intact. Masses of pink sporodochia of a Fusarium were present on one sweet-clover girdle, but this fungus may have been saprophytic. The Arkansas specimen, a portion of which was deposited in the pathological collections at Washington, D. C., shows pycnidia of what appear to be *Diplodina medicaginis*.

BACTERIAL LEAF SPOT

The bacterial leaf spot, caused by *Bacterium trifoliorum* and described by Jones and others (1923) on various species of Trifolium, was found once on red clover and once on white clover in this State, but it appears to be of little importance.

FUSARIUM ROOT ROT

Various unidentified Fusaria have been isolated during the course of these investigations from rotted clover crowns and roots in the State. Neuweiler (1928) states that *Fusarium trifolii* causes wilt and death of clover plants in Switzerland. Selby and Manns (1909) hold that *Fusarium roseum*, causing the so-called scab of cereals, is able to attack clover plants in Ohio. Young (1924:63) in the same State isolated many Fusaria from rotted clover roots and after making inoculation experiments he concludes

that "some of these Fusaria are weak parasites which are able to infect clover but require the cooperation of other deleterious agencies to induce fatal results."

DOWNY MILDEW

Peronospora trifoliorum, the cause of downy mildew, was found once on red clover in the State, but it occurs more extensively on alfalfa (Stewart and others, 1908:394). Patel (1926) found the disease limited to alfalfa and yellow trefoil.

DODDER

Dodder occurs, but it is relatively uncommon on clover and alfalfa. Stewart and others (1908:361 379) give notes on this disease in the State.

RHIZOCTONOSIS

According to Stewart (1919:175), Rhizoctonia causes the leaves of white clover under moist conditions to rot. *Rhizoctonia crocorum* (Pers.) DC. may occur on clovers and alfalfa in the State, since it is known to occur here on potatoes (Plant Disease Reporter, Supplement 34:149. 1924) but it has not been seen by the author. Recently Buddin and Wakefield in England (1927) have connected the fungus with *Helicobasidium purpureum* (Tul.) on fairly good evidence. Stevens and Wilson (1912) report a Rhizoctonia as causing a root rot of clover in North Carolina.

SCLEROTINIA STEM CANKER

Sclerotinia stem canker was collected once in New York at Phelps in 1901 by Stewart (1908:387). Gilbert and Bennett (1917), who have published a good American account of this disease, believe that Stewart's fungus is *S. trifoliorum*. The writer made a thorough search for this disease during June, 1928, but discovered no trace of it in this State.

THIELAVIA ROOT ROT

Thielavia basicola has been found to cause root rot on red clover in New York (Burkholder, 1916:104). It sometimes affects clover in Connecticut also, according to Gilbert (1909:20). It was not found by the author during the seasons he has been in the field.

BLACK STEM

Black stem recently reported by Valleau and Fergus (1929) occurs destructively on red clover in New York. It may be mistaken easily for anthracnose, but no fruiting structures of a pathogene have ever been seen. The cause of the disease is unknown.

DISEASES OF GRASSES

HETEROSPORIUM LEAF SPOT OF TIMOTHY

Suscepts

Timothy alone has been found to be a suscept of the Heterosporium leaf spot. Although a similar disease has been observed on red top, it probably is not identical. Gregory (1919:578) states that orchard grass is never infected when inoculated in parallel with timothy. The writer has obtained infection only on timothy in the greenhouse. Inoculations upon Kentucky blue-grass, red top, and orchard grass have uniformly failed of positive results, although timothy in the same series of experiments was always infected.

The disease

The name *Heterosporium leaf spot* is used in this paper to distinguish this from other leaf-spot diseases on timothy. Gregory (1919:576) first applied this name.

History and range

This disease apparently was first described in a short paper by Gregory in 1919. He states that he found timothy plants affected by this disease in many parts of New York, and he is of the opinion that it probably occurs wherever timothy is grown (1919:576). The only foreign report of the disease since its discovery is a doubtful case mentioned by Jørstad (1924:17).

Economic importance

Gregory (1919:576) believes that the actual damage is negligible, but states that this is the most common disease of timothy in New York. The writer has observed several cases in which the disease was so severe that 100 per cent of the plants in the field were more or less spotted. Although the loss from leaf spots is hard to estimate, the damage probably is not negligible.

Symptomatology

Once the identity of the spots, which occur only on leaves, has been recognized, they are readily distinguished thereafter. The spots are small, oval in shape, with the long axis parallel with the veins. They vary from 0.5 to 1 millimeter in width and from 1 to 4 millimeters in length; occasionally they are larger. The center of the spot is characteristically light mauve

colored with a narrow dark madder violet or dark nigrosin violet margin (Ridgway, 1912). Sometimes the purple color is faded, so that the margin appears dark brown. At other times the reverse condition obtains, in which case the purple color becomes so dark that it appears almost black. When spots become numerous, the tissue between them is yellowed, and severely affected leaves are killed.

Etiology

Name, history, and classification of the pathogene. The causal agent in this disease is the fungus *Heterosporium phlei* Greg. The original description constitutes almost the only record of the fungus. The reason probably is that the fungus rarely produces spores in the field, and this makes mycological identification difficult. Spores have never been observed in nature by the writer. The fungus is closely related to the genus Helminthosporium, from which it differs in having echinulate conidia, two- to several-septate. Helminthosporium spores are smooth and pluriseptate.

Pathogenicity. Gregory (1919:578) first completed Koch's rules of proof with *Heterosporium phlei.* The results obtained by the writer agree with those of Gregory. Several futile attempts were made to isolate the fungus during the summer of 1926, but it was successfully isolated later by sterilizing bits of affected leaves for five minutes in 1–1000 bichloride of mercury followed by a thorough rinsing in sterile water. Success in isolation depends upon transferring a piece of leaf bearing a spot to a tube of agar rather than planting several on an agar plate as is usually done. Thus any rapidly growing saprophyte can overrun the pathogene only in the tube in which it happens to occur. In the first isolation three tubes out of ten showed this fungus in pure culture at the end of two weeks as a minute outgrowth from the piece of leaf tissue. Single spore cultures were obtained subsequently. Oat agar is colored dark purple like the leaf spots. Inoculation with two strains produced the typical leaf spots in ten days on two-months-old timothy seedlings in the greenhouse. The fungus was reisolated from the lesions, and the typical cultural characters of *Heterosporium phlei* were produced again.

Life history. Almost no data on the life history of this fungus are reported in the literature. It seems to overwinter on the green leaves, as lesions have been observed on these in the early spring before secondary infections could have occurred.

Spores germinate readily in tap or distilled water in the laboratory on glass slides in about twelve hours, sending out a germ tube from one or both ends.

Obviously, the temperature at which spores will germinate best is a factor in incubation. Two experiments to test this were tried, the average results of which are presented in table 21 and illustrated graphically in figure 18.

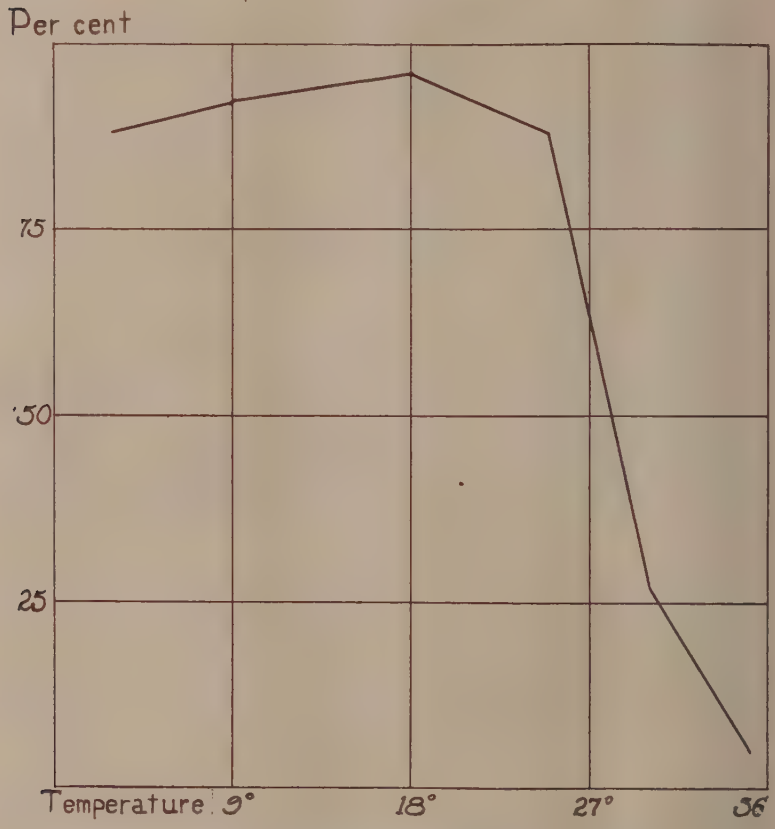


FIGURE 18. EFFECT OF TEMPERATURE ON GERMINATION OF CONIDIA OF HETEROSPORIUM PHLEI

TABLE 21. Effect of Temperature on Conidial Germination of Heterosporium Phlei

Temperature (degrees C)	Number of tests	Number of spores	Per cent of germination
3	4	1,049	87.7
9	4	965	92.1
18	2	441	97.1
25	4	948	87.5
30	2	434	27.2
35	2	457	5.0

It is evident that ordinary spring and summer temperatures are satisfactory for germination of the spores, since they seem to germinate practically as well at 3° as at 25° C. Temperatures of 30° and 35° C. appear to be too high for the best germination. More time for germination at the lower temperature levels might have allowed more germination of the spores.

In both cases the spores were allowed to remain on the slides for forty-eight hours before making germination counts. This was to make sure that all the germinable spores had grown; but as time curves for the various temperatures were desirable, another experiment was made to provide the necessary data.

It is shown in figure 18 that germination occurs equally well at 25° and 3° C., but it was predicted that the curve for 3° would show a lag behind the curve for 25°. Accordingly, a series of slides of the same spore suspension was prepared and placed in moist chambers at the two temperatures, 3° and 25°. Two slides were removed from each incubator at intervals of two hours, and the amount of germination was recorded. The results of the experiment are presented in table 22.

TABLE 22. Effect of Time upon Conidial Germination of Heterosporium Phlei at 3° and 25° Centigrade

Time	Hours elapsed	3° Centigrade		25° Centigrade	
		Total number of spores	Per cent of germination	Total number of spores	Per cent of germination
9 a.m.	0				
11 a.m.	2	No germination		No germination	
1 p.m.	4	448	2.2	580	23.6
3 p.m.	6	426	5.0	459	70.6
5 p.m.	8	262	10.3	491	85.7
7 p.m.	10	503	30.1	481	89.9
9 p.m.	12	445	45.8	446	92.6
10 p.m.	14	501	55.7	450	91.8
8 a.m.	23	541	84.0	473	89.8

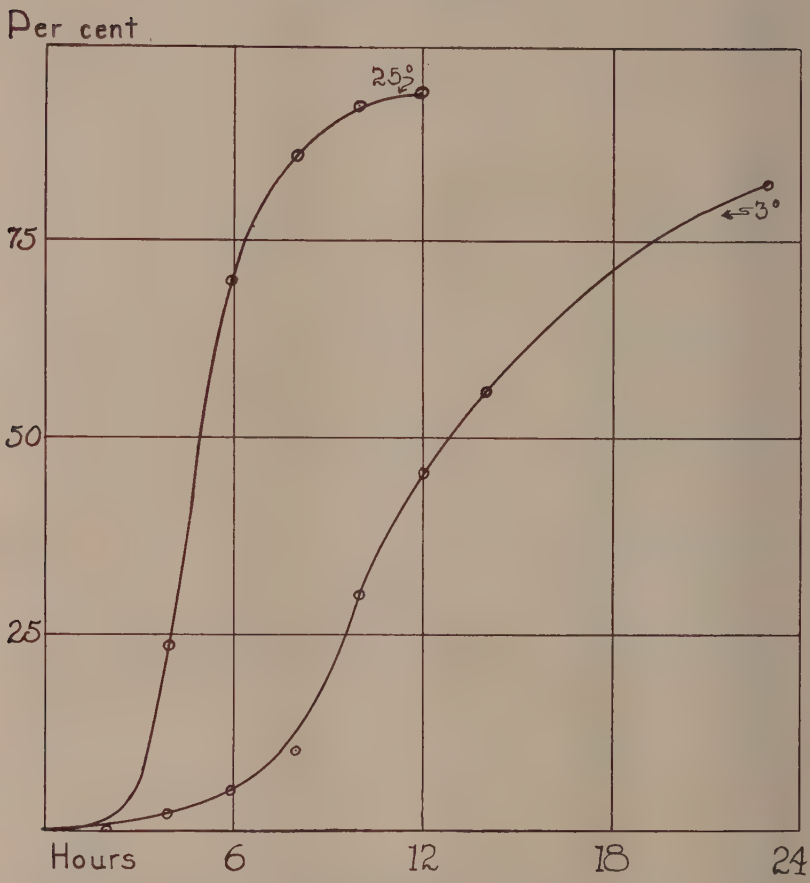


FIGURE 19. GERMINATION OF CONIDIA OF HETEROSPORIUM PHLEI AT 3° AND 25° C., SHOWING GOOD GERMINATION AT THE LOWER TEMPERATURE DESPITE A LAG IN THE CURVE

The results, as shown graphically in figure 19, are exactly those that were predicted. There is a well-defined lag in the germination of the spores at the low temperature.

Nothing is known of the mode of entrance o˚ the germ tubes except tha they appear to be able to penetrate uninjured tissues.

In one experiment a spore suspension in distilled water was atomized on two-months-old timothy seedlings in pots in the greenhouse, and the pots were placed in a moist chamber for forty-eight hours. Seven days later small light-brown spots had begun to appear on inoculated leaves. Within two days the spots had become more typical, showing the light straw-colored center and the purple margin.

Although no definite data are at hand on secondary cycles, the spores doubtless are carried by rain and wind from plant to plant during the growing season, giving rise to new lesions.

Control

Since the fungus has been shown to germinate practically as well at 3° as at 25° C. if time enough be allowed, it should serve as a most satisfactory organism with which to test the common opinion that the action of sulfur depends upon its volatilization at high temperatures. Barss' statement (1926) that " hot weather is believed to be essential in getting a thorough control with sulfur dust since the heat causes the sulfur to volatilize and give off fumes which kill the fungus and its spores " has been cited already under the discussion of powdery mildew of clover. To test this hypothesis, spores of *H. phlei* were germinated in contact with sulfur in two experiments each in duplicate at various temperatures to see if the toxicity of the sulfur varied with the temperature. The results of these two experiments appear in table 23.

TABLE 23. Effect of Temperature on the Action of Sulfur on Spores of Heterosporium Phlei

Experiment no.	Temperature (degrees C.)	Treatment	Number of spores	Per cent of germination
1	3	Check	455	90.3
		Kolodust	428	5.6
		300-mesh dusting sulfur	419	18.8
	9	Check	417	93.2
		Kolodust	382	12.4
		300-mesh dusting sulfur	417	16.2
	18	Check	451	97.1
		Kolodust	403	11.1
		300-mesh dusting sulfur	389	44.0
	25	Check	422	92.4
		Kolodust	408	7.3
		300-mesh dusting sulfur	464	31.6
2	3	Check	532	83.7
		Kolodust	445	2.9
		300-mesh dusting sulfur	435	37.0
	25	Check	473	89.8
		Kolodust	425	5.4
		300-mesh dusting sulfur	501	31.0

Bank's colloidal sulfur dust prevented germination completely at all temperatures and hence is not included in the table. The differences in germination in Kolodust were not great at any temperature. The same is true of the germination in 300-mesh dusting sulfur, but the latter invariably allowed more germination than the former.

It appears that sulfur is as active at one temperature as at another. Kolodust shows less variability in this respect than does 300-mesh dusting sulfur. This is probably due to the fact that the 300-mesh sulfur is near the border line of toxicity. It has been pointed out previously that dusts at the lower limit of toxicity cause variations in germination in different experiments because of difficulties inherent in the method of application of the dusts.

Caution must be observed in drawing conclusions from the data. One could not say, for example, that sulfur does not kill by volatilizing, but, if volatilization is responsible, it proceeds sufficiently rapidly at 3° C. to inhibit the germination of *Heterosporium phlei*, and that an increase in temperature from 3° C. to 25° C. does not change the degree of toxicity.

If all the germinations in the checks and in the two sulfurs be considered as accurate indications of their true values, then a summary of the results with sulfur is obtained as shown in table 24.

It is readily evident from the table that Kolodust is superior to 300-mesh dusting sulfur in efficiency, but that both are rather effective in

TABLE 24. SUMMARY OF THE EFFECT OF SULFUR ON CONIDIAL GERMINATION OF HETEROSPORIUM PHLEI

Experiment no.	Temperature (degrees C)	Per cent of germination			
		Check	300-mesh dusting sulfur	Kolodust	Bank's colloidal sulfur dust
1	Room	90.1	6.8	11.4	2.7
1a	Room	90.4	3.8	11.5	2.8
2	3°	92.5	18.4	5.8	0.0
2a	3°	88.2	19.2	5.4	0.0
2b	9°	94.6	19.5	10.0	0.0
2c	9°	91.8	12.9	14.9	0.0
2d	18°	98.1	64.5	11.2	0.0
2e	18°	96.2	23.5	11.1	0.0
2f	25°	93.9	40.9	7.1	0.0
2g	25°	90.9	26.3	7.5	Trace
3	3°	81.6	37.4	3.3	...
3a	3°	85.8	36.5	2.4	...
3b	25°	90.0	28.5	5.5	...
3c	25°	89.6	33.6	5.3	...
4	Room	96.3	....	13.1	...
4a	Room	95.9	....	27.5	...

Check, $E_m = 91.6 \pm 0.76$.
Kolodust, $E_m = 9.56 \pm 0.046$.
300-mesh dusting sulfur, $E_m = 26.6 \pm 2.84$.

inhibiting germination. Kolodust probably would give protection against the disease in the field.

TIMOTHY RUST

Suscepts

The results of a great number of cross inoculations between timothy, cereals, and wild grasses reported in the papers of Eriksson (1894), Kern (1909), Arthur (1909), Johnson (1911), Pammel and King (1912), Mercer (1914), Stakman and Jensen (1915), Stakman and Piemeisel (1916 and 1917) have been compiled. The plants shown to be infected more or less readily from inoculations with urediniospores from timothy are: *Avena elatior, Avena fatua, Avena sativa, Bromus tectorum, Dactylis glomerata, Elymus virginicus, Festuca elatior, Hordeum jubatum, Hordeum vulgare, Lolium italicum, Lolium perenne, Poa compressa,* and *Secale cereale.* The following plants have been inoculated by investigators from time to time without positive results: *Agrostis alba, Agropyron occidentale, Elymus canadensis, Elymus robustus, Holcus lanatus, Hystryx hystrix, Phalaris arundinacea, Poa pratensis, Triticum vulgare,* and *Mahonia aquifolium.* As a result of their studies, Stakman and Piemeisel conclude that the following plants may be infected in nature with timothy rust: *Dactylis glomerata, Festuca elatior, Festuca pratensis, Koeleria cristata,* and *Phleum pratense.*

Whether the barberry, *Berberis vulgaris,* is susceptible to the rust is still debatable. Eriksson (1894) did obtain infection once under uncontrolled conditions, but apparently no one has been able to confirm the experiment. As far back as 1864 Settegast observed that timothy can grow very near rusted barberries without becoming diseased (1864:384). It should be pointed out here that timothy is susceptible not only to timothy rust but also to *Puccinia coronata,* oats-form and lolium-form (Melhus, Dietz, and Willey, 1922:225 and 227); and to *Puccinia graminis avenae* (Stakman and Piemeisel, 1916:816) but is not susceptible to *Puccinia graminis agrostis* (Stakman and Piemeisel, 1917:470), to *Puccinia graminis tritici* (Carleton 1899:54, Stakman and Piemeisel, 1917:470), nor to *Puccinia graminis hordei* (Stakman and Jensen, 1915:213).

The general consensus of opinion among investigators seems to be that no timothy varieties are immune to timothy rust, although much resistance is indicated by some of them. Clark (1910:431) observed that "some plants show great susceptibility to the attacks of this fungus, being completely covered with the pustules. On the other hand, a few plants have been observed which are very nearly immune."

As a result of their study of varietal susceptibility, Hayes and Stakman (1919:68) say that "the striking fact is that the Cornell selections show a high percentage of resistant plants, while the Minnesota selections are very susceptible."

The disease

Since the name *timothy rust* is adequate for the purpose, it is adopted in this bulletin.

History and range

Settegast in 1864 remarked that timothy is not affected with rust when growing in juxtaposition to barberries (1864:384). In the United States, Trelease (1885:131) first recorded timothy rust in 1885. It was not reported again in America until 1906 when it "became epidemic in the experimental plats at the Arlington Experimental Farm" (Johnson, 1911:7). In 1907 it was first discovered in New York in the Cornell timothy plantings at Ithaca. It was abundant the same year in Pennsylvania and Indiana (Webber, 1912:352). From that time the fungus has been found in many places in the United States and Canada. Mercer (1914:20) remarks that "the spreading and destructiveness of the disease has been little short of phenomenal."

Arthur (1920:297) gives the distribution of this rust from Nova Scotia and Maine to Louisiana and from Virginia to Washington, California, and Manitoba. The disease appears to be ubiquitous in the United States and Canada. The Sydows (1904:785) report the disease in Europe from Germany, Austria, Denmark, and Sweden. Stapledon and others (1922: 16) find it in Wales, and Madame Teterevnikova (1927) states that it occurs in a severe form on timothy in Russia.

Economic importance

Timothy rust is a destructive disease. Webber (1912:352) in an early report on the disease in New York State says, "Observations made every year for the last five years in various parts of the State have shown that rust is the most serious disease affecting timothy." During 1926 to 1928, however, this condition did not obtain in New York. It may be that timothy was more subject to the disease when it first became epiphytotic during the first decade of this century. At any rate, it was far overshadowed by the Cercospora leaf spot in 1928. Miss Sampson reports a great reduction in seed yield in Wales as a result of rust. She says that in 1922 the weight of 1000 seeds was reduced from 0.39 to 0.19 gram and in 1923 the weight of an equal number of seeds was reduced from 0.43 to 0.31 gram. Johnson and Haskell (1920:75) report an estimated loss of 2 per cent of the crop in Minnesota in 1919. Cook and Helyar (1915:6) note that "the timothy rust is also occasionally found in New Jersey but cannot be considered of importance."

Symptomatology

Symptoms do not play a very important part in the diagnosis of this disease. The leaves may be of a nondescript yellow color as they are killed

by copious infections, but when only one or two small infections occur scattered over the leaf, little or no discoloration appears adjacent to them. Sometimes there is a narrow zone of yellow surrounding each pustule. Barker and Hayes (1924:367) note that " very sharp hypersensitive areas do not appear in timothy. Resistant plants give small pustules which are not confluent. Marked chlorosis is usually, but not always, associated with resistance."

Timothy rust is most noticeably evident on leaves and culms in the urediniospore stage as yellow-brown, characteristically elongated pustules protruding through the epidermis which is visible with a hand lens as a whitish partially sloughed off scale. The oblong linear sori may approach 5 millimeters in length. In some instances they are confluent. The uredinial sori on timothy culms are shown in figure 20. Note the scurfy appearance due to the bits of epidermis which have been pushed aside by the developing sori.

Etiology

Name, history, and classification of the pathogene. Even after more than twenty-five years the question that Eriksson asked in 1902 seems to persist, " Ist der Timotheengrasrost eine selbständige Rostart oder nicht?" In 1894 Eriksson and Henning (1894:140) described a new species, *Puccinia phlei-pratensis*, on timothy, differentiating it on the basis of the lack of an aecial stage on Berberis. In 1902 Eriksson asked the question just quoted and maintained that it should be a separate species. He thinks (1902:198) that *Puccinia phlei-pratensis* may have arisen originally from *Puccinia graminis* and " that it has differentiated gradually over a long period of time into a distinct species, in so far that it has lost its aecium-producing capacity, but that it has retained its inner nature so that it can return weakly to oats and rye " Klebahn (1904:235), Sydow and Sydow (1904:784), and Evans (1907:447) likewise retain Eriksson's name.

Kern (1909) maintains that Eriksson's one successful inoculation of barberry is worth mo e than all the negative results since obtained, so that he advocates calling the fungus a physiological form of *Puccinia poculiformis* (*graminis*). A year later (1910:418), however, he retracts somewhat and says, " Rather than calling it a race, physiological species or form species, it might be better to consider it a variety or subspecies since it does, as previously pointed out, possess some slight morphological differences from the typical form, particularly in the small aecial cups and the more delicate uredinial mycelium." Johnson (1911), Pammel and King (1912), and Mercer (1914) use the name *Puccinia phlei-pratensis*. Stakman and Jensen (1915) avoid naming the fungus in their paper reporting inoculation experiments, but Stakman and Piemeisel (1916) call it

FIGURE 20. UREDINIAL SORI OF PUCCINIA PHLEI-PRATENSIS ON CULMS OF TIMOTHY

Puccinia phlei-pratensis. The same authors the next year, however, establish *Puccinia graminis phlei-pratensis* comb. nov. (1917), and yet they use the name *Puccinia phlei-pratensis* in many places in the same paper. In being thus inconsistent, they admit that the relation of the rust to *Puccinia graminis* is still probably debatable, but they favor including it as a biologic form of *Puccinia graminis* (1917:482).

Arthur, in the *North American Flora* lists the fungus as *Diaceoma poculiforme* (Jacq.) Arth. (*Puccinia graminis* Pers.), but in his new book on plant rusts (1929:328) he calls it *Puccinia graminis phlei-pratensis*. Stakman (1922:251) reverts to Eriksson's original name in summing up the diseases of cereal and forage crops in the United States in 1921, for there he lists the timothy rust organism as *Puccinia phlei-pratensis* Eriks. & Henn.

It is obvious from this brief review of the literature that the name of this fungus has been changed about considerably by taxonomists of the rusts. In view of the following facts, the author favors retaining the name *Puccinia phlei-pratensis:* (1) the fungus has produced neither pycnia nor aecia under controlled conditions; (2) the uredinial mycelium is appreciably smaller than that of *Puccinia graminis;* (3) teliospores are rare; (4) the fungus overwinters in the uredinial condition in northern latitudes; (5) no evidence of biologic specialization has been obtained; (6) the uredinia are distinctively linear instead of oval or elliptical; (7) the urediniospores are predominantly pyriform rather than oval, elliptical, or elongate; (8) the author who made it a biologic form of *Puccinia graminis* subsequently calls it *Puccinia phlei-pratensis*.

Pathogenicity. Certainly the array of inoculations already cited under the discussion of the suscepts leaves no doubt of the pathogenicity of *P. phlei-pratensis*.

Only one paper seems to exist reporting inoculations with collections of the rust from different places to see if any specialization occurs within the species. Barker and Hayes (1924:369–370) inoculated various clonal lines with collections of timothy rust from different parts of the United States and Canada. "No definite evidence of biologic specialization of *Puccinia graminis phleipratensis* was found." In view of the rather recent work of Craigie (1927) this may be due to the absence of an aecial suscept.

Life history. That the fungus overwinters as perennial mycelium is hardly to be doubted. Eriksson and Henning (1894:141) first advanced evidence in support of this hypothesis. Johnson (1911) developed uredinial sori from overwintered green leaves and he also determined that viable urediniospores may be found after they have passed the winter. Hungerford (1914:337) gives supporting evidence for this opinion.

More than twenty-five separate experiments by the writer on the toxicity of chemicals in which many thousands of spores were examined have revealed some interesting characteristics of the process of spore germination.

The range of conditions suitable for germination seems narrow in many respects. Urediniospores refuse to germinate well if they are scraped from the sori, whereas, if they are washed from the leaf with a stream of distilled water from a wash bottle, germination is good. This may be due to the fact that the latter method removes mostly the more mature spores and does not injure them (Doran, 1922).

The urediniospores either have a specific gravity of less than unity or they are not easily wettable, for they float on the surface of the water drops. When the drops are placed in van Tiegham cells, the spores collect in the lower part of the drop, and then their tubes intermingle so freely that the percentage of germination is almost impossible of determination. If, on the other hand, raised drops are placed on slides in moist chambers, the spores float down to the periphery and appear as a ring of tangled tubes and spores after germination has occurred. Spores that happen to be immersed in the drops do not germinate very well. This indicates a high oxygen requirement.

The best results have been obtained by placing the drops of spore suspension on clean glass slides, and, after the spores have germinated, removing the surplus water with a pipette. The surface tension tends to separate the ring of spores at the edge of the drop so that the individuals are more readily discerned.

Difficulty has been experienced also in allowing too long a time to elapse before counting. After twenty-four hours the tubes become hyaline at the point of emergence from the spore and possess an index of refraction essentially the same as that of water. For this reason, counts mus be made as soon as possible after the last spore has germinated. Usually this is within three hours at room temperature. Thus it will be seen that an incredibly short length of ime is necessary for complete germination. Another advantage which accrues from making the counts early is that the tangling of tubes is at a minimum.

Even when all these conditions have been complied with, germination of the checks is sometimes still low. A few of the reasons for this are: (1) imperfectly cleaned slides, (2) staling products in the moist chambers, (3) toxic tap water (distilled water should be used), (4) parasitized spores, and (5) immature or overripe spores.

No satisfactory time curve has been obtained, but certainly it must be relatively steep since the peak is reached within three hours.

Temperature has a minimum effect upon germination if sufficient time be allowed. Germination is a slower process at low temperatures. A glance at the graph in figure 21 shows, however, that germination after eighteen hours is just as good at 3° C. as it is at 26° C. Unfortunately a time curve for finding the minimum time for each point was not made on the same day for each temperature chamber. Had that been done, and

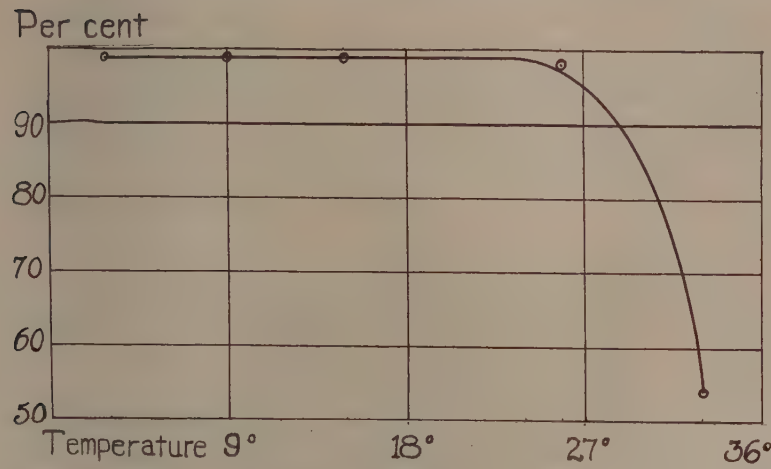


Figure 21. Effect of temperature on germination of urediniospores of Puccinia phlei-pratensis

Eighteen hours allows as good germination at 3° as at 26° C.

had a curve been plotted of the germination in each chamber when the maximum germination was attained in the best one, a more exact idea of the effect of temperature could be obtained. It is significant, nevertheless, that complete germination occurred even at 3° C. within eighteen hours. Apparently 33° C. is too high for the best germination.

Evans (1907:446–448) studied the disease cytologically and found that a fine, delicate germ tube runs along the leaf surface and swells into a thin appressorium at a stoma. The protoplasm then passes into the substomatal vesicle and the appressorium dries up.

Evans (1907:447) says also that the substomatal vesicle "gives rise at one end to one infecting hypha only, from which the others subsequently arise." The first septum is formed about the third day when a haustorium begins to develop from the swollen hyphal tip as a little tube which penetrates the cell wall and expands within the cell itself. As the body of the haustorium increases in size, the proximal swollen portion becomes empty and the nuclei pass into the distal end. Sori appear within eight to twelve days.

Urediniospores produced in the primary cycles initiate the secondary cycles which follow each other at approximately fortnightly intervals. Teliospores may or may not develop later in the season (Eriksson and Henning, 1894:140).

Epiphytology

Some epiphytological factor as yet unknown must govern the appearance of timothy rust during the season, since it appears most plentifully as a rule only late in the summer. During 1926, 1927, and 1928 it has occurred but little before the first cutting of hay is made, but it is found frequently on the growth following the first cutting. Kern (1910:417) found the first timothy rust collected in Indiana in a low, wet spot on rich soil. As is the case with wheat rust, the urediniospores are undoubtedly transported by the wind.

Control

A large number of laboratory experiments with various dusts were made to determine what would be likely to serve best in the field as protectants.

Effect of sulfur. In more than ten different tests Bank's colloidal sulfur undiluted always inhibited germination completely except on one slide where about 8 per cent of the spores did show the initial germination. The stock Kolodust always allowed a small amount of germination, but the tubes were dwarfed and distorted considerably, so that infection probably would not have been produced by them had this been on plants instead of glass slides.

A typical data sheet for the three sulfurs is given in table 25.

TABLE 25. EFFECT OF SULFUR DUSTS ON UREDINIOSPORE GERMINATION OF PUCCINIA PHLEI-PRATENSIS

Treatment	Total number of spores	Per cent of germination
Check	453	97.8
Bank's colloidal sulfur dust	521	0.0
Kolodust	483	5.6
300-mesh dusting sulfur	497	16.9

Many other tests showed similar results for the sulfurs[4]. From these data one would be led to conclude that diseases probably can be controlled in the field with sulfur dust.

In the spring of 1927 the effect of sulfur was tried in the field upon timothy left in a two-years-old field after the clover had been killed out. Eight contiguous plots, 1 by 3 rods in size, separated by alleys 1 yard wide, were dusted checkerboard fashion with Kolodust, making four dusted and four undusted plots in the experiment. The first application was made on May 3, just as the first new green leaves were beginning to appear. Subsequent applications were made on May 13, May 29, and June 19.

[4] The germ tubes produced in contact with various dusts are drawn with a camera lucida in figure 22. Note that the germ tubes which develop in the sulfur dusts are gnarled and distorted, so that they probably are too weak to get through the stomata.

An artificial epiphytotic was started June 4 by surrounding each plot with ten pots of severely rusted plants from the greenhouse. The epiphytotic was fairly well established on the check plots by the time the timothy was harvested on July 11. The rust on the dusted plots appeared less abundant to the eye at the time. The hay was cut just as most of the plants passed the blooming stage. Green weights of the plots are given in table 26.

TABLE 26. Results of Field Dusting for Timothy Rust

(Green weight)

Dusted, 167 pounds	Check, 145 pounds
Check, 177 pounds	Dusted, 202 pounds
Dusted, 188 pounds	Check, 177 pounds
Check, 178 pounds	Dusted, 206 pounds

Observation of the field and of the data as well, reveals that the land increases in fertility towards the south (foot of table 26). The only adequate method of calculating the data therefore is by the pairing of contiguous east-west plots, which gives a mean difference of 21.5 ± 2.47 which is highly significant. This difference shows a gain of 11.8 per cent by the use of the sulfur dust. These figures agree with the laboratory results. Unfortunately, a lack of adequate facilities for further field work precluded a continuation of these studies in 1928.

Effect of copper. In the spring of 1927 a great many laboratory tests of copper-lime dust diluted in lime were made. Dusts containing the following percentages of metallic copper were used: 6.8, 3.4, 1.7, and 0.34. The data obtained are to be found in table 27.

TABLE 27. Effect of Copper-Lime Dust on Urediniospore Germination of Puccinia Phlei-Pratensis

Per cent of copper	Number of tests	Number of spores	Per cent of germination
None + (check)	9	2,209	88.3 ± 2.48
6.8	15	3,969	9.5 ± 1.76
3.4	18	4,292	17.3 ± 2.52
1.7	24	6,211	17.8 ± 1.25
0.34	19	4,691	17.85 ± 2.25

It is obvious that timothy-rust urediniospores are very resistant to copper because they germinated approximately 10 per cent even in the presence of 6.8 per cent of copper. Another fact that the data emphasize is that there is no essential difference in germination whether the spores were in contact with one dilution of dust or the other. Except possibly for the 6.8-per-cent dilution, the copper content appears to have nothing to do with the results. Since the dusts were composed of monohydrated copper sulfate and hydrated lime, the lime was the only substance that could be acting to equalize the dusts, because the copper content was variable. The lime content varied somewhat, too, but since the least amount was about 93 per cent, its relative fluctuation was not great. This conclusion at the time of the tests was not without foundation because several tests with lime alone showed marked reduction in germination.

A subsequent experiment seemed to clinch the argument. In this test duplicate slides on which approximately 600 spores were counted for each treatment, 7.2-per-cent copper-lime dust and lime alone, showed an average of 16.3 per cent germination in the copper lime and 19.3 per cent in the lime alone. A reference to the drawings in figure 22 shows that germ tubes

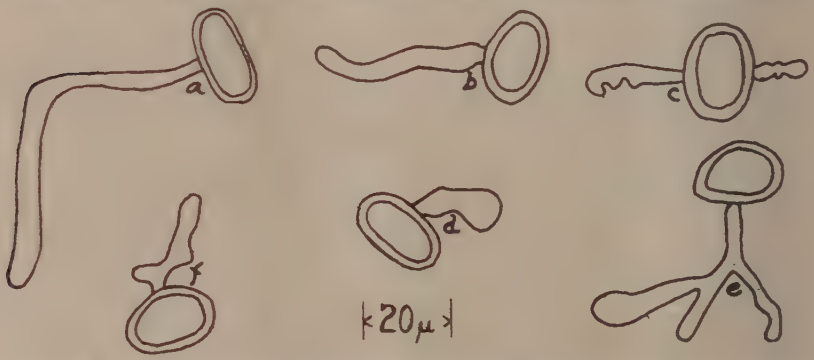


FIGURE 22. GERMINATING UREDINIOSPORES OF PUCCINIA PHLEI-PRATENSIS, SHOWING THE TOXIC EFFECTS OF FUNGICIDES

a, Check; *b*, in copper-lime dust; *c*, *d*, and *e*, in 200-mesh dusting sulfur; *f*, in Bank's colloidal sulfur dust

growing in the presence of copper-lime dust appear normal. It would be of interest to find an inert carrier for the monohydrated copper sulfate which would not of itself affect the spores, in order to determine just how toxic copper really is. Bentonite alone in one experiment reduced the germination approximately one-third. Incidentally, in a single experiment the spores germinated 16.6 per cent in 4–4–50 bordeaux mixture also.

The copper sulfate might be diluted in water to determine the minimum point of toxicity.

No studies with copper-lime dust in the field have been made, but the evidence in hand indicates rather clearly that sulfur dust at least may be depended upon to control timothy rust in the field and that copper-lime dust if not altogether inefficient, would certainly be less satisfactory than the sulfur.

Investigators have maintained that the only hope of combating the trouble successfully lies in the development of disease-resistant plants. Timothy varieties do vary in their susceptibility to the disease, as indicated under varietal susceptibility, and, since no specialization of the fungus seems to occur, the development of disease-resistant varieties of timothy is not such an unpromising method as it seems to be in the breeding of resistant varieties of wheat.

Barker and Hayes (1924:370) show that resistance or susceptibility seems to depend upon a single factor pair. "Resistance appears to be dominant, and a close approximation to a 3:1 ratio was obtained in the progeny of self-fertilized resistan plants." Proytcheff 1928 confirms the conclusions of Barker and Hayes in general, but he thinks that he has some evidence of modifying factors influencing the degree of resistance. The nature and the number of these factors were not determined.

CERCOSPORA LEAF SPOT OF GRASSES

Suscepts

According to the writer's collections, Cercospora leaf spot attacks timothy, orchard grass, red top, blue-grass, and *Alopecurus geniculatus* in New York. In published records the following additional species of grasses are recorded as suscepts: *Bromus uniloides*, *Hordeum jubatum* (Spegazzini, 1911:436), *Bromus marginatus*, *Bromus sitchensis*, *Hordeum nodosum*, *Elymus glaucus*, *Elymus robustus* (Johnson and Hungerford, 1917: 69), *Alopecurus fulvus*, *Oryza clandestina*, *Milium effusum*, *Poa nemoralis*, *Glyceria fluitans*, *Glyceria plicata* (Schroeter, 1908:498), *Anthoxanthum odoratum*, *Arrhenatherum elatius*, *Avena elatior*, *Avena sativa*, *Brachypodium* sp., *Cynosurus cristatus*, *Glyceria spatilis*, *Poa compressa*, *Poa trivialis*, *Secale cereale* (Lindau, 1907:794), and *Avena bulbosa* (Tracy and Earle, 1895 b:115).

The disease

The malady, for which the name *Cercospora leaf spot* is proposed, probably is the most important disease of timothy in the State at the present time. It is serious also on orchard grass. It seems to have been recorded first in America by Trelease (1887). The writings indicate that it is very widely distributed, at least in America and Europe.

Symptomatology

Probably the most characteristic symptom is circular to elliptical, small, purple-brown spots, with brown to gray centers, dispersed over the leaf surface. These spots seem to be more in evidence when the disease occurs on mature tissues, for they are most frequently conspicuous late in the season. These spots on timothy leaves may be seen in figure 23. These leaves show chiefly, however, the long aniline to sulphine yellow streaks (Ridgway, 1912) which result from elongation of the spots parallel to the midrib. An incipient stage of the latter type of spotting is shown at *A* in figure 23.

The disease appears to be slightly different early in the season on orchard grass as the following notes on symptoms, taken in May, 1928, indicate: The spots occur on leaf sheaths and blades where they tend to congregate on the midrib, resulting in a cracking of the tissues at that point. They appear at first as small elongate, hydrotic areas, but they enlarge rapidly, soon becoming one to two centimeters long or even longer. The hydrotic center early in the morning when the dew is on is deep olive gray (Ridgway, 1912), but upon drying it becomes deep dull gray. This is surrounded by a band of pecan brown, and this in turn by a narrow strip of maize yellow. The spots occurring toward the proximal end of the leaf cause it to turn yellow over the entire length.

All the symptoms described were noted on the spots that bore the minute black conidial stromata. These are arranged in rows in the center of the spots parallel to the veins of the leaf.

Etiology

Name, history, and classification of the pathogene. Fuckel in 1863 described the causal organism as *Scolecotrichum graminis* in *Fungi Rhenani Exsiccati* (1863:134). Subsequently many species of Scolecotrichum as well as varieties of *S. graminis* were described on grasses. Recently, however, von Höhnel (1924:6–7) has shown that the genus Scolecotrichum probably is invalid. He places this fungus in *Passalora graminis* (Fckl.) Höhn. and includes *S. compressa* Allescher (Hedwigia 35^2:34. 1896) and *S. graminis* (Fckl.) var. *nana* Sacc. (Annales Mycologia 3:515. 1905.) as synonyms, saying that they pertain to the young condition of the fungus.

Von Höhnel seems to have overlooked *Cercospora graminicola* (Tracy and Earle, 1895 a:179) in his disposition of the fungus. Type material of this fungus, which was kindly lent to the writer by Dr. Charles Chupp, was compared with various American collections of *S. graminis* and specimens in European exsiccati for which the writer is indebted to Dr. John A. Stevenson of the Office of Mycology, Bureau of Plant Industry, Washington, D. C. The identity of the two fungi is obvious. The genus

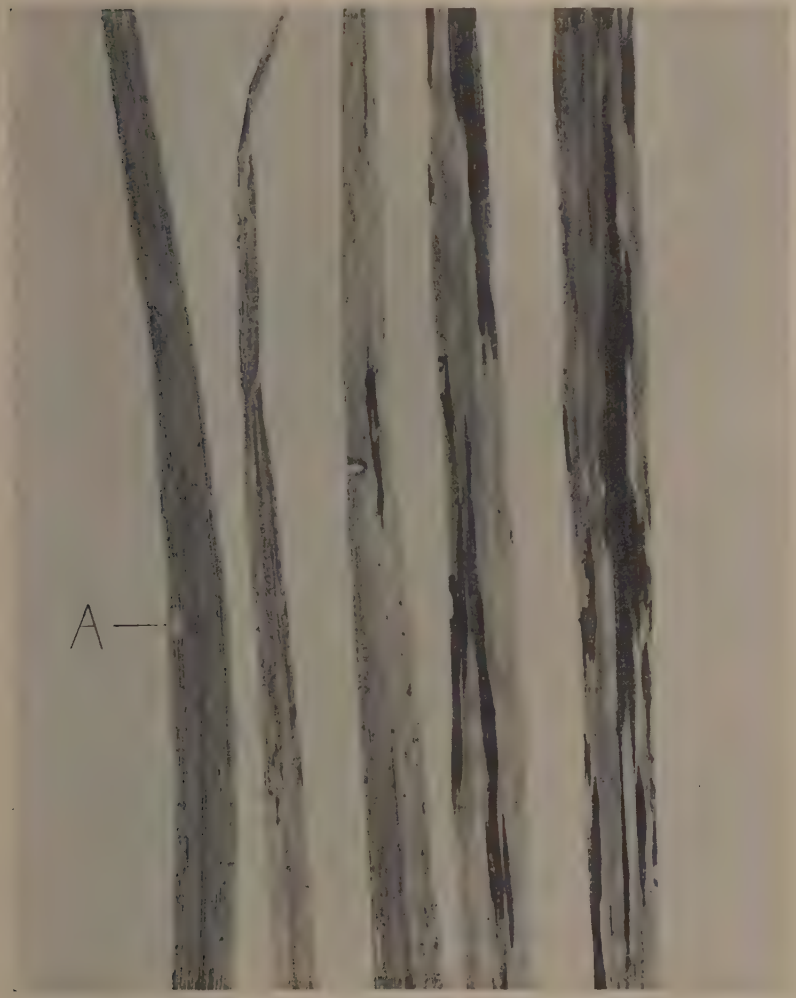


Figure 23. Lesions caused by Cercospora graminis on leaves of timothy
Long yellow streaks result from the elongation of spots (A) parallel to the midrib

Cercospora seems a more logical place for the fungus than the genus Passalora, because the latter has many-septate conidiophores, while these are chiefly continuous or rarely septate. In the absence of type material of *S. graminis*, of course, no disposition can be considered as final; nevertheless the fungus can be included without reasonable doubt as *Cercospora graminis* (Fckl.) comb. nov. The following names are listed as synonyms with the authority for considering them as such inclosed in parenthesis after each:

Scolecotrichum graminis Fckl. (writer, 1929)
Passalora dactylina Pass (Lindau, 1907:794)
Passalora graminis (Fckl.) Höh. (writer, 1929)
Scolecotrichum graminis var. *nana* Sacc. (Von Höhnel, 1924:6)
Scolecotrichum compressum Allesch. (Von Höhnel, 1924:7)
Cercospora graminicola Tracy & Earle (writer, 1929)

Species that are closely related to if not identical with *Cercospora graminis* are *Scolecotrichum graminis* var. *avenae* (Eriksson, 1891:28); *S. sticticum*[5] (Berk. and Br.) (Oudemans, 1874:315); *S. graminis* (Fckl.) var. *brachypoda*[6] (Spegazzini, 1911:436); *Passalora punctiformis* and *P. hordei* (Otth, 1868).

The identity of these species cannot well be determined in the absence of the type material. The similarity of their descriptions evidently indicates that further investigation might result in their incorporation as synonyms of *Cercospora graminis*. *Azozma punctum* Lacr. has sometimes been included as a synonym, but von Höhnel (1924:6) states that this is an error.

Fuckel (1869:107) listed *Scolecotrichum graminis* as the conidial stage of *Sphaeria recutita* Fries (1822:524), although as Schroeter said (1908: 498), "aber wohl ohne genügenden Grund." Von Höhnel (1924:6) referred this fungus to *Carlia recutita* (Fr.) Höh.

Single spore cultures of *C. graminis* produce what are undoubtedly perithecial primordia on sterilized wheat grains, but no method of treatment has yet induced these to form asci and spores. Exposing the cultures to ultra-violet light and to outside conditions that were successful for *Pseudoplea trifolii* was of no avail in this case. In the spring of 1928 perithecia of a fungus provisionally identified at the time as *Mycosphaerella* sp. were found on overwintered leaves of orchard grass bearing conidial fructifications of *C. graminis*. The ascospores were biseriate, hyaline, and one-septate with one cell frequently somewhat larger than the other. Ten spores measured 7.2 to 9.0 μ by 23.0 to 32.4 μ in size, thus corresponding with conidia of *C. graminis*. An attempt was made to get a pure culture

[5] Oudemans' figure is typical of *C. graminis* and the spores measure 40μ long according to Lindau (1907: 794).
[6] The description of this fungus might well pass for that of *C. graminis*.

rom these spores, but they failed to shoot upward to agar. This fungus may be the same as that described by Fries, but his description is meager. *Leptosphaeria culmorum* Awd., which occurs on various grasses, such as quack grass, timothy, and orchard grass, is probably not the perfect stage of *C. graminis*, since single ascospore cultures from these three grasses are in no wise comparable with single spore cultures of *C. graminis*.

No information is available on the pathogenicity of the fungus, its life cycle, or measures for its control.

HELMINTHOSPORIUM LEAF SPOT OF BLUE-GRASS

Suscepts

Kentucky blue-grass suffers from Helminthosporium leaf spot in the field, and orchard grass has been inoculated successfully in the greenhouse with a spore suspension. Four cereals, wheat, barley, rye, and oats, were inoculated in the greenhouse. No infection occurred, although blue-grass inoculated at the same time did become infected.

The disease

History and range

It appears that in New York blue-grass is almost universally affected with this disease, since specimens have been collected in practically every planting examined during 1926, 1927, and 1928. Drechsler (1923:687) has collected it at many stations on Long Island, as well as in Maine, Massachusetts, Connecticut, Virginia, Maryland, Illinois, and Wisconsin. The disease was first mentioned by Drechsler (1922:35). Monteith (1925) apparently is the only other author who makes any reference to this malady.

Economic importance

The economic importance of blue-grass lies in its use on lawns or golf courses as well as in pastures and meadows. Since this disease rarely interferes with the beauty of the grass to the casual observer, it probably is of minor importance in spite of its ubiquity. The disease does remain, however, as an incipient menace to blue-grass, because it was observed to make one lawn very unsightly under moist conditions.

Symptomatology

The disease occurs on leaf blades and sheaths. The first symptom to appear following inoculation is a minute hydrotic spot which soon enlarges and assumes a characteristic oval shape, at first uniformly reddish or

brownish in color. Later a grayish center is differentiated. Lesions vary in size from almost invisible points up to 10 to 15 millimeters in length and 1 to 3 millimeters wide, depending upon the width of the leaf.

Although in the field the spots vary considerably in color, still the general aspect is one of a light-colored center surrounded by a margin of reddish, purplish, or brownish hue. Comparison with Ridgway's color standards (1912) give: for the center, hazel, pale ochraceous-salmon, light grayish vinaceous-lilac, pale vinaceous-lilac, brownish drab, and pale brownish drab; for the margin, Sanford's brown, Prout's brown, yellow ochre, vinaceous-brown, taupe brown, anthracene purple, blackish brown (1), and dull purplish black. Typical foliar lesions are shown in figure 24.

Infections in the greenhouse do not appear to show as dark shades as in the field, especially on the margin. Here colors such as eugenia red, van Dyke red, vinaceous-brown, or Indian lake prevail. Seedlings growing in inoculated soil in the greenhouse have been observed to damp off at the ground line after developing the characteristic reddish lesions.

On leaf sheaths the spots are not so regular as on the blades. Large, irregular, brown blotches usually obtain here, so that the greatest damage probably occurs on the sheaths at the bases of the leaves.

Etiology

Name, history, and classification of the pathogene. Helminthosporium vagans Drechs., the causal agent in this disease, was discovered by Charles Drechsler and was first reported in 1922 (1922:35). A year later (1923) he described the fungus as a new species. Drechsler states that the fungus resembles *Helminthosporium sativum*, P. B. K., the cause of foot rot of wheat, in having thick-walled, dark-olivaceous spores, but it differs from the latter in having larger conidiophores, in growing slower, and in sporulating only meagerly on potato dextrose agar. *H. sativum* spores, when they germinate, produce germ tubes only from the terminal cells, but in *H. vagans* they arise from the central segments as well.

Pathogenicity. The writer isolated the pathogene, but no spores were found in cultures on sterilized wheat kernels, oat agar, potato agar, or solidified synthetic media of Coons (1916) and Leonian (1924).

The pathogenicity of the fungus was demonstrated in two trials by planting seed in soil contaminated artificially with a pure culture. The organism was reisolated from the reddish brown lesions produced on seedlings which frequently damped-off. The fungus was reisolated moreover from infections that were obtained by using mycelium covered with moist cotton as inoculum.

Figure 24. Lesions caused by Helminthosporium vagans on leaves of blue-grass

After extensive investigations a method of obtaining conidial production in culture was perfected.[7] The spores thus produced were used for inoculation trials in which the typical leaf symptoms were produced within nine days on blue-grass. The pathogene was reisolated from these lesions.

Physiologic specializat on. Christensen (1922) and Stevens (1922) showed that *Helminthosporium sativum* P. B. K. causing the Helminthosporium disease of cereals was a composite species of several physiologic strains. Ch istensen in a later paper (1926:12) separated several forms by their reactions on culture media, rate of growth, thermal relations, amount of sporulation, zonation, the readiness with which they mutate, and lastly by their pathogenicity.

A test for the presence of such strains in the species *Helminthosporium vagans* Drechs. was undertaken in a small way by the writer. During the summer of 1927 several isolations of the fungus were made from blue-grass in as many localities in New York State as possible. The culture number, the place of collection, and the date of these isolations, are shown in table 28.

TABLE 28. ISOLATIONS OF HELMINTHOSPORIUM VAGANS

Culture no.	Place of collection	Date of collection
127	Ithaca	June 21, 1926
128	Ithaca	June 21, 1926
231	Elba	July 2, 1927
232	Ithaca	June 22, 1927
242	Horseheads	July 31, 1927
244	Mecklenburg	July 31, 1927
251	Ithaca	August 12, 1927
271	Enfield Glen	August 17, 1927
272	Sennett	September 7, 1927

Single spore cultures of all the strains were made except for number 271 which was made as a tissue planting and never wa converted to a single spore culture.

Tests of the reaction of the various strains to four culture media were made. Coons' (1916) synthetic medium with 2-per-cent agar, oatmeal

[7] The fact that the fungus was found to sporulate by placing transfers on oat or potato agar on a north window ledge according to the method of Jones (1916), encouraged the writer to investigate the stimulus to spore production, the results of which are described briefly. It appears that light is the factor usually limiting spore production in the laboratory where the cultures were usually kept in darkness or semi-darkness. Transfers in quadruplicate to oat agar were made in March, 1928, for all the possible combinations of the following conditions: sealed, unsealed, covered with black paper, uncovered, on north window ledge, and in the greenhouse. Spores were produced in abundance within two weeks only in those tubes that were uncovered whether sealed or not. No spores could be found in those from which light had been excluded. The sealed tubes remained moist, while the unsealed ones dried somewhat, indicating that moisture is unimportant. Ultra-violet light was not a factor, inasmuch as it will not pass through the glass of the test tubes. Direct sunlight is not important, because the tubes on the north window ledge were in diffuse light only. Alternating temperature does not appear to exert much effect, since spores were produced in a subsequent experiment when the transfers were placed in constant temperature incubators heated by incandescent bulbs.

agar,[8] cornmeal agar, and potato agar, were prepared and plates were poured with 20 cubic centimeters of each. For each isolation triplicate plates of each medium were inoculated as uniformly as possible in the center with a piece of agar containing mycelium. All of the plates were placed on a laboratory shelf under identical conditions. The diameter of the thalli to the nearest millimeter was determined daily at the same hour, by making two measurements at right angles to each other. Plates containing occasional contaminations were discarded. Measuring the thalli every day was unsatisfactory because of the marked daily variation under the conditions of the experiment.

The data are summarized in table 29. These same figures would have been obtained by measuring the thalli at the end of the experiment instead of daily.

TABLE 29. Average Daily Increase in Diameter (in Millimeters) of Thalli of Various Strains of Helminthosporium Vagans

Medium	Culture no.								
	231	232	251	127	272	271	128	242	244
Potato	2.66	2.13	2.11	2.88	2.58	3.21	3.54	13.1	11.67
Oat	5.21	5.25	6.27	4.87	3.48	3.50	5.47	12.0	11.33
Cornmeal	4.39	4.04	5.48	4.54	4.85	3.00	5.65	12.6	11.90
Coons'	....	....	....	2.39	2.95	....	3.30	....	.....

The table indicates that 231, 232, 251, and 127, as well as 271 and 272, may be placed in a group which grow at about the same rate. Isolation 128 might conceivably be placed in this group, but it grows uniformly somewhat faster than do the others. Cultures 242 and 244 are in a class by themselves and differ from each other in that 242 grows slightly the faster.

From observations of color reactions and general appearance made at the conclusion of the experiment, the following cultures were grouped together as probably representing a single physiologic form: 127, 231, 232, 251, 271, and 272. The color of this group by reflected light on potato agar ranges from blackish green gray (Ridgway, 1912) to Sanford brown in the center. There is a narrow apricot-orange margin which is sharply defined. It will be recalled that these colors appear in the symptomatology complex. On oat agar the aerial mycelium is somewhat more prominent in the center, where it ranges from a blackish brown to an olivaceous black. Oat agar appears to be the best differential medium for the forms. On cornmeal agar differences are not striking. The mycelium is much thin-

[8] The media were prepared as follows: Coons', 10 cubic centimeters each of M/5 magnesium sulfate plus 7 H_2O, potassium acid phosphate, asparagin, and maltose, per liter; oat, 35 grams of 3-minute oat flakes and 14 grams of agar per liter; cornmeal, 30 grams of Quaker cornmeal and 14 grams of agar per liter; and potato, 210 grams of peeled potatoes and 18 grams of agar per liter.

ner, tending in early stages to be almost invisible within an internal ring about 3 or 4 millimeters from the edge. This gives the effect of a white halo around the young thallus. The mycelium is a dark ivy green on cornmeal agar.

Culture 128 stands definitely alone, since it produces an abundance of "white islands," which appear almost like minute somewhat feathery white Clavarias in the plate. These structures, which are arranged in concentric circles, are made up of closely compacted, large, barrel-shaped hyphal cells that branch and fray out slightly at the distal ends. They are from 2 to 4 millimeters high. The submerged mycelium has a velvety olivaceous black appearance which fades to a medal bronz at the edge, fringed with white. After the culture is two or three weeks old, the blackish submerged mycelium tends to grow up and over the white islands, dulling their appearance.

Cultures 242 and 244 also appear greatly different from any of the others and different from each other. Culture 242 is Hiathi gray uniformly over the plate on oat agar. A few small white clumps, quite unlike those of 128, occur scattered over the plates. There is a small, sharply defined central portion of lilac gray which changes to dawn gray on the margin. Cornmeal agar separates out this strain because the mycelium here is a dark grayish olive in the center, grading off to a light olive gray at the edge instead of the usual dark ivy green. As the description indicates, the mycelium of this form is much lighter than of any of the others.

Culture 244 resembles 242 but it is somewhat darker and shows a white fluffy center on all agars. It is dark olive gray around the edge on potato agar, storm gray on oat agar, and deep olive on cornmeal agar. The submerged mycelium is somewhat rhizomorphic on the last medium.

On Coons' synthetic medium the fungus grows very slowly. That medium is useless for differentitation.

After having found these differences in culture, the next logical objective was to determine whether differences in pathogenicity occurred, but a lack of time prevented this from being carried to completion.

Mutation. It seemed desirable also to determine if *Helminthosporium vagans* mutates in culture. No sign of sector mutation was observed in any of the strain work outlined previously until suddenly toward the end a single "white island" similar to those described under culture 128 arose from the otherwise blackish mass in one of the plates of oat agar of culture 271. Upon transferring one of these carefully to another plate of oat agar, as 271 *d*, it developed almost precisely like culture 128 except possibly that the submerged mycelium tended to be more nearly pink. White islands developed profusely. Three subcultures of white islands failed to give increased general whiteness of the culture. A hint of dark color in the submerged mycelium remained.

Culture 271 also produced another sector mutation of a reddish brown color, which was designated 271 *a*. This was distinctly different from the original culture. Subsequently in plate culture the white-island mutation, 271 *d*, produced a definite sector 271 *da*, which corresponded with the original 271. Then the reddish brown mutation, 271 *a*, formed two additional sectors. One of these, 271 *aa*, was definitely pink, and the other reverted to the 271 type. Still later a sector was found that formed heavy rhizomorphic branches on cornmeal agar and was distinctly of a dark yellow green, 271 *ab*. It probably corresponds to the original form. One other mutation from culture 271 arose. It was of a gray-green color resembling very much culture 244. Cultures 272, 232, 231, and 251 were then transferred to oat-agar plates, and the pink form corresponding with 271 *aa* arose from each.

Using mycelium in lieu of spores, these so-called mutations were inoculated into blue-grass to see whether they would retain their characteristics after assuming a parasitic relationship. The most pathogenic mutation was culture 271 *da*, the white-island form. It induced a violent case of the disease within four days. Of less virulence were 271 *aa*, the pink form, 271 *ca*, another white-island form similar to 271 *da*, and 271 *ba* which resembled culture 244.

Reisolation was successful with cultures 271 *da*, 271 *ca*, 271 *ba*, and 271 *aa*. The white islands of the first two, as well as the rapid growing and gray characters of 271 *ba*, came out unchanged, but the pink form of 271 *aa* was not recovered.

It seems that mutation occurs in the species but that many of them are relatively unstable, as evidenced by the reversion in culture. This seems to be especially true of culture 271 *aa*, the pink form. Pink color is more or less evident in lesions in nature, and tends to occur somewhat in all the cultures, especially on oat agar. Pink color also tends to form in the presence of contaminations, so that it is possibly a response to environment. Culture 271 *aa* arose as a direct sector and bred true except for occasional reversions to the original condition. Although in reisolations it was recovered as the original form, this does not prove that the mutation 271 *aa* is not a definite entity, since it might merely have reverted again to the original condition within the suscept.

Reversion did not occur within the suscept in the case of the two white-island mutations, 271 *da* and 271 *ca*, which arose independently. These were reisolated without change. The tendency for white islands to form in test-tube cultures of 231, 232, and 251 was also observed. These have never been seen in culture 127 or culture 272. The white-island condition seems to be as stable as, or more stable than, what is called the *typical condition*.

Culture 271 *ba*, since it was reisolated unchanged, is probably also relatively stable. The chief point of the mutation studies is that, of the

four physiologic strains postulated from the cultural studies, three were produced by mutation corresponding to cultures 128 and 244, and the typical condition, as represented by culture 231, was secured as a reversion from a mutation. A strain corresponding with culture 242 was not obtained by mutation, but a new one, 271 *aa*, appeared.

Effect of temperature on cultural characters. As one of the corallaries of the studies of the effect of media, culture 127, representing the typical fungus, was grown in triplicate plates containing 20 cubic centimeters each of Coons' synthetic medium at various temperatures. The diameters of the thalli were measured daily to the nearest millimeter. A duplicate experiment of shorter duration revealed the same results.

Both experiments demonstrate that the best growth occurred at 25° C., with a minimum somewhere below 3° and a maximum between 30° and 35° C.

The data from the first experiment are presented in the graphs shown as figures 25 and 26. In figure 25 is shown the growth curve based on the final diameter of the thalli. The peak is very sharp. The daily growth rate at the various temperatures is plotted in figure 26.

Life history. Although the complete life history of *Helminthosporium vagans* is not known exactly, yet enough data have been accumulated to warrant their inclusion here.

In the spring two primary sources of inoculum exist. To judge from a single year's observations, the fungus lives over winter in lesions on living leaves. Also, mycelium and spores were overwintered in test tubes of oat agar on a north window ledge where the temperature reached as low as 5° to 10° F. on several occasions. In the spring the agar had liquified from freezing, but transfers gave typical cultures of the fungus. The vegetative mycelium is capable therefore of living all winter, at least under the conditions of this experiment, and of growing again in the spring. This indicates the possibility of its living in refuse or in living leaves of blue-grass. No perfect stage has yet been found, although immature perithecia which may belong to this fungus have been observed on overwintered infected leaves. Conidia from dead leaves probably serve as the chief inoculum.

An experiment was made in duplicate to test the effect of temperature on spore germination. Counts after twenty-four hours revealed that the spores had germinated 98 per cent or better in all temperature chambers from 3° to 35° C. Obviously the fungus will germinate over a very wide range of temperature if sufficient time be allowed. It is interesting that germination occurs well at 35° C., whereas no appreciable growth occurs when plate cultures are incubated at that temperature. At 9° and at 3° C. there is a hint of shortening of the tubes, but that is a retardation of growth rather than a reduction of germination.

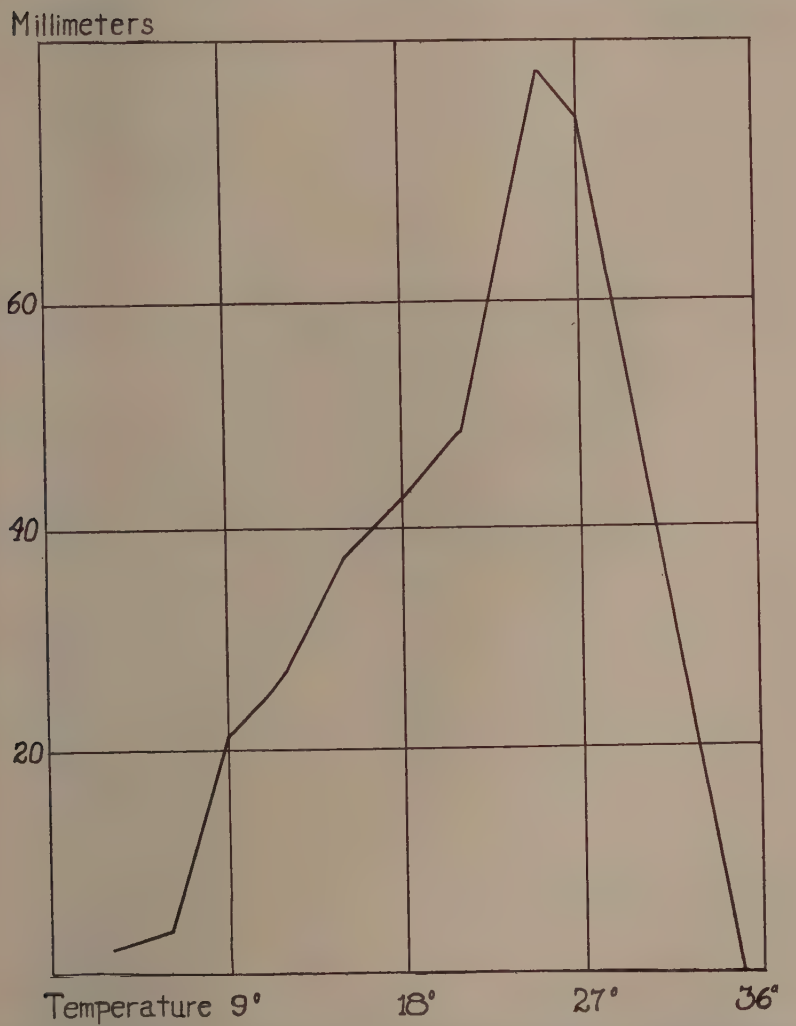


Figure 25. Effect of temperature on the growth of thalli of Helminthosporium vagans in triplicate plates of 20 centimeters of Coons' solidified synthetic medium

Diameters of the thalli in millimeters

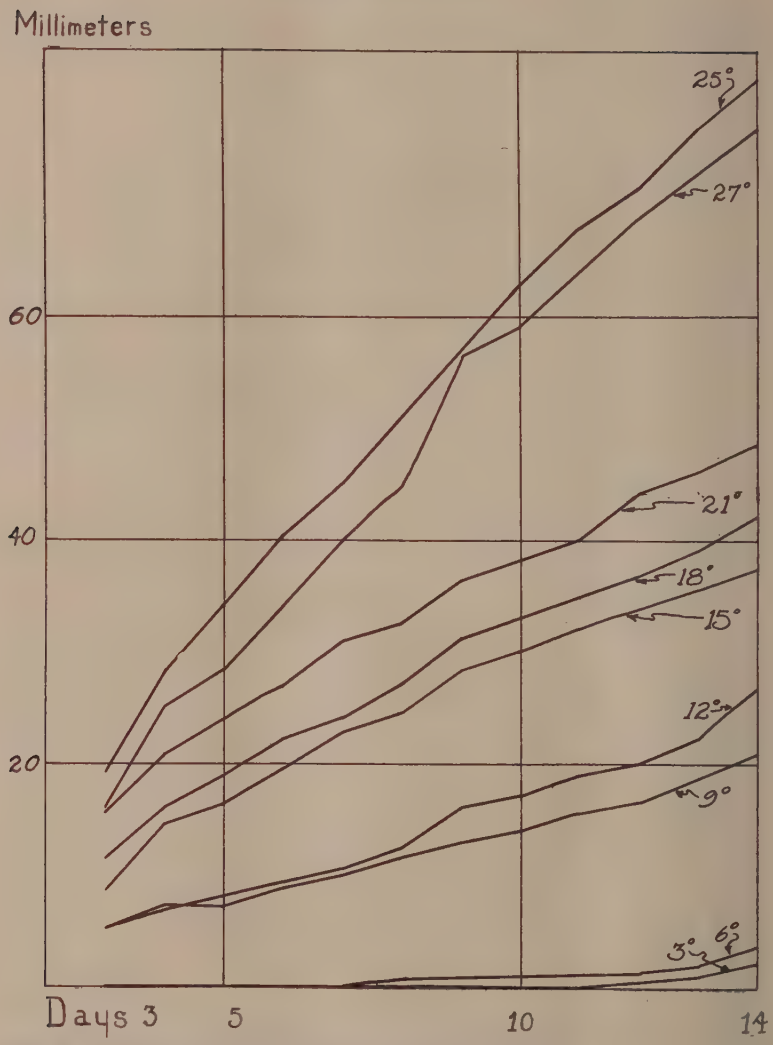


FIGURE 26. AVERAGE DAILY GROWTH OF THALLI OF HELMINTHOSPORIUM VAGANS IN TRIPLICATE PLATES CONTAINING 20 CENTIMETERS OF COONS' SOLIDIFIED SYNTHETIC MEDIA AT VARIOUS TEMPERATURES

The optimum could best be found by determining the minimum time necessary for complete germination at each temperature, but the time consumed probably would not be warranted. Instead, a series of slides

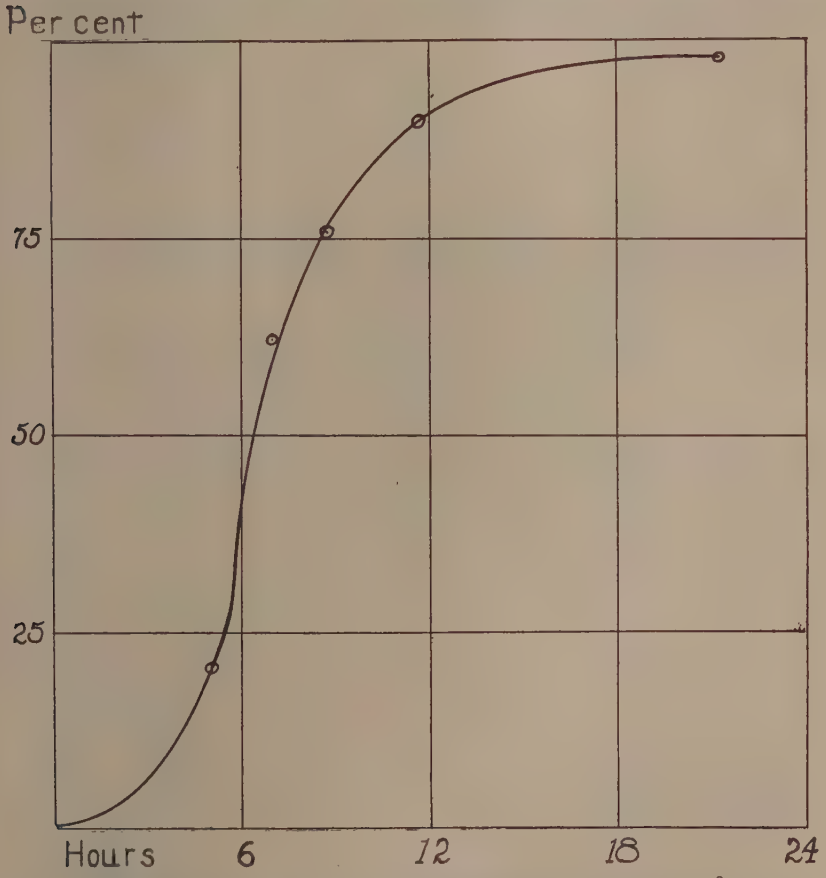


Figure 27. Germination of conidia Helminthosporium vagans at 25° C.

with spore suspensions were placed at 25° C. and a time curve was procured. The data are given in table 30 and are presented graphically in figure 27.

TABLE 30. EFFECT OF TIME ON CONIDIAL GERMINATION OF HELMINTHOSPORIUM VAGANS AT 25° C.

Hours elapsed	Total number of spores	Per cent of germination
5	391	20.5
7	388	58.7
8¾	407	75.7
11¾	426	90.2
21¼	359	97.4

The first visible hydrotic symptom may be observed within four days in the greenhouse. At the end of a week following inoculation, the typical symptoms are well developed.

Since spores are found in greatest abundance on severely diseased or dead material, the fungus probably pursues a distinct saprogenic existence, although little is known about it.

Many secondary cycles may occur in one season, because only from one to two weeks need elapse between the generations of spores.

Control

All the studies on control have been directed toward testing copper- and sulfur-dust protectants.

The effect of sulfur upon the germination of the spores of the pathogene was tested in three experiments and the effect of copper-lime dust in four, each of which was made in duplicate. The data from these experiments are summarized in table 31.

TABLE 31. SUMMARY OF THE EFFECT OF COPPER AND SULPUR DUSTS ON CONIDIAL GERMINATION OF HELMINTHOSPORIUM VAGANS

Treatment	Total number of spores	Per cent of germination
Check	1,793	97.8
7.2-per-cent copper-lime dust	645	7.7
3.6-per-cent copper-lime dust	1,159	58.8
Bank's colloidal sulfur dust	1,012	89.1
Kolodust	635	99.3
300-mesh dusting sulfur	691	91.1

Even the most toxic form of sulfur, Bank's colloidal sulfur undiluted, had little or no effect in inhibiting germination. Copper-lime dust, however, especially when used at full strength, seems to be promising for field experiments. Even the germ tubes that did emerge are abnormal, gnarled,

and stunted, indicating strong toxic effects. In figure 28 a spore germinating in the presence of 7.2-per-cent copper-lime dust is shown. This spore seems to be typical of the effect of copper. Most of the spores that germinated in this dust produced the three-branched germ tubes from the

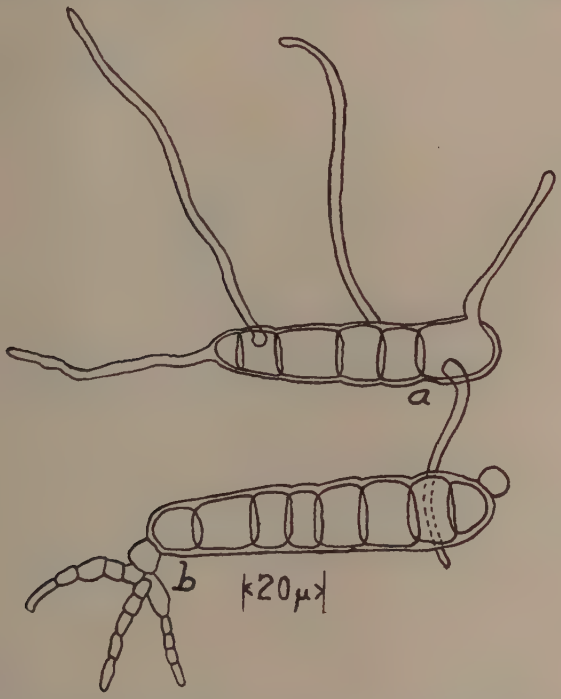


Figure 28. Germinating conidia of Helminthosporium vagans, showing the toxic effect of copper-lime dust

a, Germination in distilled water; *b*, in copper-lime dust

terminal cells and almost never from any of the central segments. The sketch of spore germination in distilled water may well represent the germination in sulfur dusts.

These tests indicate that commercial copper-lime dust applied under moist conditions will probably control the disease satisfactorily by preventing spore germination and penetration, and that any sulfur treatment is likely to be ineffective in the field.

The two diluents, lime and talc, were tried alone to see whether they were exerting any toxic effects. They were used only once, but this was sufficient to demonstrate their non-toxicity, as shown in table 32.

TABLE 32. EFFECT OF DILUENTS ON CONIDIAL GERMINATION OF HELMINTHOSPORIUM VAGANS

Treatment	Total number of spores	Per cent of germination
Check	422	99.2
Lime	429	98.0
Talc	434	99.0

HELMINTHOSPORIUM LEAF SPOT OF ORCHARD GRASS AND RED TOP

Helminthosporium leaf spot on red top appears first as small spots of Dresden brown (Ridgway, 1912) surrounded by a salmon-colored zone which fades into the normal green of the leaf. The spots are small at first, but they rapidly elongate in the direction of the long axis of the leaf, forming streaks which finally result in the death of the affected organ. The fungus fructifies abundantly on old lesions, appearing as a black fuzz. The development of the malady on orchard grass is less well known, but the fungus was isolated once from yellowed areas on leaves of this suscept.

The causal agent was identified provisionally as *Helminthosporium triseptatum* Drechsler (Drechsler, 1923:686), since the spores were almost without exception two- or three-septate. Drechsler believes that it is a saprophyte. Spores have never been seen in culture on Leonian's medium and they are rare on oat agar and potato agar, but they are produced abundantly on sterilized wheat kernals at room temperature. The mycelium is black and otherwise characteristic of Helminthosporia in culture.

Conidia thus produced in cultures isolated from orchard grass were germinated at the following temperatures: 3°, 9°, 15°, 21°, 25°, 30°, 35° C. in duplicate tests. The percentage of germination ranged from 98.6 at 3° C. to 99.5 at 35° C.

These counts which were made after forty-eight hours indicate that temperature within this range really has little effect upon germination if time enough be allowed. It is interesting, however, that the fungus germinated well at 35° C., while the spores of most of the other fungi investigated were injured at this temperature.

The results of two fungicide experiments are given in table 33.

Copper-lime dust appears promising for the control of this disease. The average high germination in 3.6-per-cent copper-lime dust diluted in lime is of interest, but standard 20–80 copper-lime dust probably would have inhibited germination completely.

TABLE 33. Effect of Sulfur and Copper-Lime Dusts Upon Conidial Germination of Helminthosporium Triseptatum

Treatment	Number of tests	Number of spores	Per cent of germination
Check	4	840	98.9
300-mesh dusting sulfur	4	869	98.2
Kolodust	4	855	97.5
Bank's colloidal sulfur dust	4	882	96.5
3.6-per-cent copper-lime dust	4	907	33.8
Lime alone	2	412	99.7
Talc alone	2	432	99.2

TIMOTHY SMUT

Scant attention in this investigation was devoted to timothy smut because of the excellent discussion of the disease by Osner (1917). An attempt was made to culture the smut, using the method of Christensen and Stakman (1926:981) by which they immersed corn-smut spores in 1-per-cent copper-sulfate solution and removed them at intervals up to seventy-two hours and made streak plantings with them on potato agar. No success crowned this effort probably because a period of after-ripening is necessary for the germination of these spores, as pointed out by Davis (1924 b). Davis (1928) has published a note on physiologic specialization of the fungus.

SILVER TOP

Sporotrichum poae Pk., described originally by Stewart and Hodgkiss (1908), causes what they call *silver top* on blue-grass, orchard grass, and timothy. The disease is rather common in June as the grasses are heading. It frequently accompanies the killing of the heads, which stand out prominently among the green ones. Several attempts to culture the fungus failed. Stewart, however, did culture it.

POWDERY MILDEW

Powdery mildew of grasses is common, but it seems to play a minor rôle in the meadow-crop economy in this State. Sulfur will keep it easily in check. To discuss in detail the enormous amount of work of Reed, Salmon, and others on specialization of the fungus is outside the scope of this paper.

PIRICULARIA LEAF SPOT OF FOXTAIL

Piricularia grisea, the cause of piricularia leaf spot of foxtail, (*Setaria lutescens* (Wieg.) (Hub.), is a common and a destructive disease in the State. The spots, watersoaked at first, are unmistakable. Later there is an irregular margin on old spots of raw umber color (Ridgway, 1912) and a center of light neutral gray to pallid purplish gray. In some cases the spots occur on leaf sheaths.

OTHER DISEASES

Professor F. C. Stewart of the New York State Agricultural Experiment Station at Geneva, New York, gave the writer a note regarding Rosen's (1924) bacterial disease of foxtail, which he had found near Geneva in 1919. He sent material to Rosen, who confirmed the diagnosis but remarked that the spots are different from those usually found in Arkansas in being larger and more regular in outline and somewhat more conspicuous in color. The head smut of Setaria caused by *Ustilago neglecta* Niessl. is common.

The discussion of diseases of other grasses found incidentally in meadows would occupy too much space in this paper in proportion to their importance. Miss Sampson (Stapledon and others, 1922) has discussed the diseases of miscellaneous grasses to a limited degree as they occur in Wales. Although the writer has been unable to obtain the publication in the Cornell library, there appears to be an extensive discussion of grass diseases by Bunting (1928) as they occur in the Gold Coast of Africa.

DISCUSSION AND SUMMARY

This investigation was undertaken because of the lack of easily accessible information concerning diseases of meadow crops in the State, crops which have a greater total valuation than that of any other single crop in the State.

The chief contributions of this paper are: (1) the development of a relatively simple technic for estimating loss in the field from leaf spots of meadow crops; (2) estimates of losses induced by these leaf spots in New York in 1928; (3) the symptomatological characterization of the various diseases; (4) a review of the nomenclatorial tangles to determine the most logical name for certain of the pathogenes; (5) observations and experiments upon life histories; and (6) provisional determination of the possibilities for field control of the diseases by dusting.

When an investigator sets himself to work on the diseases of a crop, he is confronted immediately with the desirability of knowing which are the most important diseases and what the losses are that are caused by each. To ascertain the losses from stem and foliage diseases is difficult but is of peculiar significance where the foliage is the most valuable portion of the plant, as is the case with most meadow crops. The method for determining losses presented herein, and which is based upon an "index of infection" procured from a careful survey of almost 200 fields in the State, gives some measure of the absolute loss in addition to rating the diseases according to their relative economic importance. The method, however, probably gives too conservative an estimate of destructiveness because it does not measure the subsequent effects which defoliation produces. With this method, however, a measure of loss in individual fields based upon the percentage of diseased leaves may be obtained.

Since it is not always easy for those who are only indirectly concerned with meadow-crop diseases to distinguish the various maladies in the field, a rather full discussion of symptoms is given for each.

The names of meadow-crop pathogenes in many instances are sadly in need of revision. A careful mycological study has been made of those considered in this paper, in order to clarify this situation, but the correct name for some of them still remains unsettled.

Many observations and experiments upon the factors governing spore germination have been made in connection with the work on several of the pathogenes, which throws new light on this phase of their life history.

It is a common assumption that the control of meadow-crop diseases by the application of fungicides is impractical and visionary. Interestingly enough, however, almost no one has tried heretofore to control these diseases with fungicides to see whether the idea really is impractical. Inasmuch as the majority of New York farmers of necessity must own a machine for the application of fungicides to such crops as potatoes or fruit, they should use that machine as much as possible in order to reduce overhead expense. Since almost all, if not all, the farmers grow some hay crop, usually timothy, clover, or alfalfa, they should find the control of the disease of these crops profitable. It may be agrued that the machine would destroy more of the crop than the increase from the applications of fungicides, but it is a well-known fact, which the writer verified during field experimentation, that meadow crops possess a remarkable power of recovery from being " trailed down " when they are young and growing vigorously. This ability to recover diminishes as the plants grow older. Wherever field tests with dusts demonstrate the feasibility of control by judicious applications early in the season, injury from driving through the crop will be of little importance.

Dusts only have been considered during the course of these investigations because of practical difficulties in the application of liquid sprays. The advantages of dusting that are of peculiar importance in this connection are: (1) the smaller weight to be transported; (2) the greater speed with which the operation is accomplished; (3) the lightness of the dust enabling it to filter down through the thick mat of leaves, giving better coverage; and (4) the less time and labor required. Of course, copper-lime dust must be applied when the leaves are wet, but, since the growth of meadow crops is more dense than most others, the leaves remain wet longer. Sulfur dust has been tested more intensively in this work than has copper-lime dust because of its greater adaptability for field work.

A grand summary of all tests made with fungicides appears in table 34. Since there are two tests in each experiment, the number of separate experiments for any particular treatment is just half the number of tests. An odd number of tests indicates that one duplicate was lost.

Other points of a more general nature concerning the fungicide tests

TABLE 34. SUMMARY OF THE EFFECT OF DUST FUNGICIDES UPON THE GERMINATION OF SPORES OF FUNGI CAUSING MEADOW-CROP DISEASES

Fungus	Check		Sulfurs						Copper lime				Lime alone		Talc alone	
			200-mesh		Kolodust		Bank's colloidal		7.2-per-cent		3.6-per-cent					
	Number of tests	Per cent of germination	Number of tests	Per cent of germination	Number of tests	Per cent of germination	Number of tests	Per cent of germination	Number of tests	Per cent of germination	Number of tests	Per cent of germination	Number of tests	Per cent of germination	Number of tests	Per cent of germination
Macrosporium sarcinaeforme	42	96.9	12	95.6	14	94.8	12	95.6	14	0.0	14	0.0	14	94.9	13	95.4
Uromyces trifolii hybridi	14	95.9	10	80.2	10	44.8	10	43.3	6	34.1	....	.....	4	85.4	....	.....
Uromyces trifolii-fallens	20	94.7	11	66.2	10	33.7	17	19.0	8	29.7	....	.....	2	97.0	....	.....
Cercospora zebrina	4	94.4	2	96.2	....	.....	4	51.4	4	0.5	....	.....	....	.....	....	.....
Pseudopleatrifolii	2	95.3	2	98.6	2	95.0	2	94.2	2	0.0	2	86.5	....	.....	....	.....
Heterosporium phlei	16	91.6	14	26.6	16	9.6	....	.....	....	.....	....	.....	....	.....	....	.....
Puccinia phleipratensis	18	88.3	2	15.0	2	5.0	10	0.0	15	9.5	18	17.3	2	19.4	....	.....
Helminthosporium vagans	7	97.8	6	91.1	4	99.3	6	89.1	6	7.7	8	58.8	2	97.7	2	99.0
Helminthosporium tri-septatum	4	98.9	4	98.2	4	97.5	4	96.5	....	.....	4	33.8	2	99.7	2	99.2

and not easily included in this table are: (1) the results of laboratory tests of fungicides vary with the dosage of material on the slide, especially if the chemical in question is diluted to or near to the point of minimum toxicity; (2) sulfur dust is no more effective at higher temperatures than at lower ones, which indicates that it does not act through volatilization or, if it does, it volatilizes with sufficient rapidity at low temperatures to be effective in the cases recorded; (3) hydrated lime apparently has no fungicidal effect on any of the fungi tested except *Puccinia phleipratensis*, but it appears to inhibit markedly the germination of the spores of this fungus, being as toxic as the copper sulfate in copper-lime dust; (4) talc has no visible effect upon spores of *Helminthosporium vagans*, *H. triseptatum*, or *Macrosporium sarcinaeforme;* (5) staling products accumulate in moist chambers used for germinating spores, so that the dishes should be washed carefully after each test.

Most of the essential facts about each disease are summarized in table 35 to avoid a lengthy summary and to facilitate reference.

Some additional facts regarding certain of the pathogenes that do not appear in table 35 may be stated briefly: (1) spore production in culture, induced in *Helminthosporium vagans* and *Pseudoplea trifolii*, by exposure to out-of-door conditions is due to the effect of light; (2) culture mutations in *Helminthosporium vagans* are stable as a rule after passage through the suscept, although work with fungous mutations reported prior to the time this work was initiated had not taken this factor into account; (3) spores of *Macrosporium sarcinaeforme* germinate equally well at all temperatures from 3° to 35° C.; (4) no cicatrice is laid down in red-clover leaves to halt the invasion of this pathogene; (5) powdery mildew has been shown to be very deleterious to red clover, contrary to most opinions; (6) by removing the water from the leaves, the fungus causes them to be deficient in photosynthetic ability, which results in stunting, the most significant symptom of powdery mildew; (7) clover extract stimulates the germination of the conidia of *Erysiphe polygoni* and *Dothidella trifolii;* (8) cool nights and warm days appear to be conducive to the spread of *E. polygoni;* (9) the pathogene causing the Cercospora leaf spot of clovers and alfalfa is *Cercospora zebrina* Pass.; (10) the writer agrees with Petrak that *Pseudoplea trifolii* is the cause of similar diseases of the clovers and alfalfa, and that *Stagonospora meliloti* also causes diseases that are similar to each other on the same suscepts; (11) girdle of alfalfa, sweet, and red clovers, and bacterial leaf spot of clovers, have been found in New York; (12) *Cercospora graminis* is the name that should be applied to the fungus commonly called *Scolecotrichum graminis;* (13) *Helminthosporium vagans* attacks orchard grass as well as blue-grass (producing mutations which pass unchanged through the suscept), consists of physiologic forms, has been induced to sporulate in culture by exposure to light, and overwinters as mycelium in living leaves.

TABLE 35. SUMMARY OF ESSENTIAL POINTS ABOUT THE MEADOW-CROP DISEASES DESCRIBED IN THIS PAPER

Disease	Name of pathogene	Plants affected	Chief symptom	Loss in the State	Fungicide sensitivity
Macrosporium leaf spot	*Macrosporium sarcinaeforme*	*Trifolium hybridum, T. pratense, T. repens, Medicago sativa, Melilotus alba*	Concentrically ringed brown spots	1.36 per cent	Copper
Alsike clover rust	*Uromyces trifolii-hybridi*	*Trifolium hybridum*	Minute, powdery, cinnamon-brown postules	0.81 per cent	Sulfur
Red clover rust	*Uromyces trifolii-fallens*	*Trifolium pratense*	Minute, powdery, cinnamon-brown postules	0.09 per cent	Sulfur
White clover rust	*Uromyces trifolii trifolii-repentis*	*Trifolium repens*	Minute, powdery, cinnamon-brown postules	0.84 per cent	Sulfur
Pseudopeziza leaf spot	*Pseudopeziza trifolii*	*Trifolium alpestre, T. arvense, T. cyathiferum, T. fragiferum, T. hybridum, T. incarnatum, T. macrodon, T. medium, T. nigrescens, T. pallescens, T. pallidum, T. pratense, T. repens, T. resupinatum, T. spadiceum*	Small, purple to brown spots, with dentritic borders; small dried apothecium in center	4.62 per cent on red clover	Copper (probably). Sulfur seems valueless
Powdery mildew	*Erysiphe polygoni*	356 species of plants, including *Medicago falcata, M. lupulina, M. sativa; Melilotus alba, M. officinalis; Trifolium agrarium, T. alpestre, T. arvense, T. filiforme, T. hybridum, T. incarnatum, T. involucratum, T. longipes, T. lupinaster, T. medium, T. minus T. monanthum, T. montanum, T. moranthum T. pauciflorum, T. pratense, T. procumbens, T. repens, T. rubens;* 12 species of Vicia, 2 of Phaseolus, 1 of Pisum	Whitish mold on leaf surface	From one-fourth to one-third of the crop in certain cases	Sulfur
Cercospora leaf spot	*Cercospora zebrina*	*Trifolium agrarium, T. alpestre, T. hybridum, T. incarnatum, T. rubrum; Medicago arabica, M. denticulata, M. hispida, M. sativa; Melilotus alba, M. officinalis*	Linear, reddish to smoky spots, usually limited by veins, especially on species of Trifolium	1.27 per cent on alsike clover 1.08 per cent on white clover 0.09 per cent on red clover	Copper
Sooty blotch	*Dothidella trifolii*	*Trifolium agrarium, T. alpestre, T. alpinum, T. elegans, T. filiforme, T. fragiferum, T. hybridum, T. lupinaster, T. medium, T. montanum, T. pratense, T. rubens, T. wormskioldii, T. reflexum, T. repens*	Black, mealy blotches	0.76 per cent on white clover 0.24 per cent on alsike clover	Not known. Sulfur seems valueless
Pseudoplea leaf spot	*Pseudoplea trifolii*	*Medicago falcata, M. hispida, M. lupulina, M. maculata, M. sativa, Trifolium hybridum, T. pratense, T. repens*	Leaves look peppered, spots usually minute	5.59 per cent on white clover 0.48 per cent on alsike clover 0.24 per cent on red clover	Copper

Clover mosaic	Undetermined	*Trifolium arvense, T. hybridum, T. incarnatum, T. pratense, T. repens, T. resupinatum; Medicago falcata, M. lupulina, M. sativa; Phaseolus vulgarus; Vicia faba*	Leaves mottled and wrinkled	Not severe	.....
Kabatiella anthracnose	*Kabatiella caulivora*	*Trifolium hybridum, T. incarnatum, T. medium, T. pratense, T. repens, T. suaveolens; Medicago lupulina; Onobrychis sativa*	Elongate, sunken, black lesions on stems. Leaves drooping and dead	Very severe	Not sensitive to sulfur
Stagonospora leaf spot	*Stagonospora meliloti*	*Trifolium hybridum, T. pratense, T. repens; Melilotus alba; Medicago lupulina, M. sativa*	Oval to circular spots on leaves. Spots: light-brown, brittle centers; dark-brown margins	1.08 per cent on white clover	Not known
Girdle	Undetermined	*Trifolium pratense, Medicago sativa, Melilotus alba*	Gray-green, sunken girdles on stems	Rare	.....
Bacterial leaf spot	*Bacterium trifoliorum*	*Trifolium hybridum, T. medium, T. pannonicum, T. pratense, T. repens.*	Inky black, angular spots on leaves	Rare	Not known
Fusarium root rots	*Fusarium* spp.	*Trifolium pratense*	Dry brown rot	Not uncommon	.....
Downy mildew	*Peronospora trifoliorum*	*Medicago sativa, Trifolium pratense*	White mycelium on water-soaked leaf spot	Rare	Probably copper
Dodder	Cuscuta sp.	*Trifolium* spp.	Yellow threads on plants	Rare	.....
Rhizoctonosis	Rhizoctonia sp.	*Trifolium pratense*	Soft rot of stems	Rare	.....
Sclerotinia stem canker	*Sclerotinia trifolium*	*Trifolium pratense*	Black sclerotia on stems	Rare	.....
Thielavia root rot	*Thielavia basicola*	*Trifolium pratense*	Roots blackened	Rare	.....
Black stem	Undetermined	*Medicago sativa, Melilotus alba, Trifolium pratense*	Elongate black lesions	Very common	.....
Heterosporium leaf spot of timothy	*Heterosporium phlei*	*Phleum pratense*	Small, oval spots, mauve-colored center, dark purple margin	Important	Sulfur
Timothy rust	*Puccinia phlei-pratensis*	*Avena elatior, A. fatua, A. sativa; Bromus tectorum; Dactylis glomerata; Elymus virginicus; Festuca elatior; Hordeum jubatum, H. vulgare; Lolium italicum, L. perenne; Poa compressa; Secale cereale*	Powdery, yellowish pustules on culms and leaves	Important on second cutting	Sulfur
Cercospora leaf spot of grasses	*Cercospora graminis*	*Agrostis alba; Alopecurus fulvus, A. geniculatus; Anthoxanthum odoratum; Arrhenathrum elatius; Avena bulbosa, A. elatior, A. sativa; Brachypodium sp.; Bromus marginatus, B. sitchensis, B. unioloides; Cynosurus cristatus; Dactylis glomerata; Elymus glaucus, E. robustus; Glyceria aquatilis, G. fluitans, G. plicata: Hordeum jubatum, H. nodosum; Milium effusum; Oryza clandestina; Phleum pratense; Poa compressa, P. nemoralis, P. pratensis, P. trivialis; Secale cereale*	Aniline to sulphine yellow streaks on blades; hydrotic at first	Most important of timothy diseases	Not determined
Helminthosporium leaf spot of blue-grass	*Helminthosporium vagans*	*Dactylis glomerata, Poa pratensis*	Oval spots, with drab center and reddish brown margin	Most important disease of blue-grass	Copper

TABLE 35 (*concluded*)

Disease	Name of pathogene	Plants affected	Chief symptom	Loss in the State	Fungicide sensitivity
Helminthosporium leaf spot of red top and orchard grass	*Helminthosporium triseptatum*	*Agrostis alba*, *Dactylis glomerata*	Terminals of leaves turn dry and brown	Not common	Copper
Timothy smut	*Ustilago striaeformis*	*Dactylis glomerata*, *Phleum pratense*, *Poa pratensis*, and others	Narrow, linear, black streaks on leaves and leaf sheaths	Unimportant	Not determined
Silver top	*Sporotrichum poae*	*Dactylis glomerata*, *Phleum pratense*, *Poa pratense*	Inflorescence turns white and blasts	Common in June on blue-grass	Not determined
Powdery mildew	*Erysiphe graminis*	Most grasses and all cereals	White mycelium on leaf surface	Common	Sulfur
Piricularia leaf spot of foxtail	*Piricularia grisea*	*Setaria lutescens*	Gray-centered spots with dark margin	Common	Not determined

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